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Design, synthesis, and biological evaluation of aminopyrazine derivatives as inhibitors of mitogen-activated protein kinase-activated protein kinase 2 (MK-2)

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This publication is dedicated to Professor Iwao Ojima on the occasion of his 70th birthday

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ABSTRACT

Several series of novel non-thiourea-containing aminopyrazine derivatives were designed based on the MK-2 inhibitors 1-(2-aminopyrazin-3-yl)methyl-2-thioureas. These compounds were synthesized and evaluated for their inhibitory activity against MK-2 enzyme in vitro. Compounds with low micromolar to sub-micromolar IC_{50} values were identified, and several compounds were also found to be active in suppressing the lipopolysaccharide (LPS)-stimulated TNFα production in THP-1 cells with minimum shift compared to their enzyme activity.

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. Over the last decade, several structural classes of compounds have been reported to be inhibitors of MK-2, among them aminocyanopyridines,[7] pyrrolo-pyridones,[8] tertrahydro-β-carbolines,[9] tricyclic indole derivatives,[10] pyrrolo-pyrimidones,[11] aminopyrazine,[12] benzothiophenes,[13] aminopyrazoles,[14] diaminopyrimidine,[15] pyrrolo[2,3-*f*]isoquinoline and derivatives,[16] spiro-δ-lactam,[17] and 2-phenylfuran derivatives.[18]

We recently reported a series of novel 1-(2-aminopyrazin-3-yl) methyl-2-thioureas as potent MK-2 inhibitors.[12] Both the aminopyrazine moiety and thiourea functionality were found to be essential for the high potency of this series of compounds. Though thioureas have been reported to be possibly useful as anti-HIV, antitumor, and antimicrobial agents,[19] the potential toxicity/carcinogenicity of thiourea function group[20] has prompted us to search for non-thiourea-based MK-2 inhibitors. In this communication, we would like to report our effort in the design, synthesis,

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and the biological evaluation of a series novel, non-thiourea aminopyrazine derivatives as MK-2 inhibitors.

The design of these aminopyrazines is based on thiourea lead **1a** and is shown in Figure 1. As reported earlier, **1a** exhibits 2.0 μM IC_{50} value in an in vitro MK-2 enzyme assay[21] and 7.9 μM IC_{50} value in a cell-based assay that measures LPS-stimulated TNFα production from THP-1 cells.[22] The novelty of the aminopyrazine structure and the relatively low shift between enzyme and cell activity are very intriguing. However, a simple replacement of the thiourea linker with an urea linker, that is, compound **1b**, completely loses the MK-2 activity (inactive at 40 μM). Several derivatives of aminopyrazine, therefore, were designed to systematically probe the SAR and identify novel, non-thiourea aminopyrazines with MK-2 potency. The scaffolds employed to replace the thiourea group included amide (**2**), sulfonamide (**3**), guanidine derivatives (**4**–**8**), heterocycles formed by joining the central thiourea moiety with the left side phenyl ring (**9**), heterocycles formed by joining the central thiourea moiety with the right side methylene group (**10**–**12**), or an urea linker with additional modifications on other parts of the molecule (**13**).

The synthesis of the amide and sulfonamide derivatives **2** and **3** was very straightforward as shown in Scheme 1. (2-Chloro-5-aminopyrazin-6-yl)methyl amine **14a**[12] was treated with acid chlorides or sulfonyl chlorides in the presence of DIEA to afford corresponding amides or sulfonamides in moderated to excellent yields.

Preparation of the *N*-alkyl and *N*-methoxy guanidine derivatives of these aminopyrazines proved to be more complicate than expected. Attempted conversion of thioureas **15**[12] to corresponding *N*-alkyl guanidines with mild Mukaiyama's reagent (2-chloro-1-methylpyridinium iodide)[23] and primary amines was not successful. Heating **15** with alkyl amines or methoxyamine in the presence of EDC at 70 °C in DMF overnight did provide the desired *N*-alkyl or *N*-methoxy guanidines **4** and **5** in low to moderated yields, along with formation of intra-molecular cyclization products **16** (Scheme 2).

The synthesis of *N*-cyano guanidines **6** is achieved following the method reported by Atwal et al.,[24] and is shown in Scheme 3.

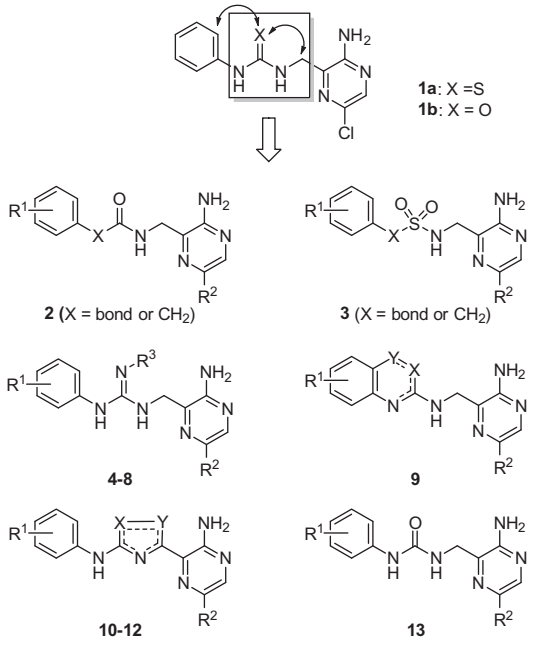
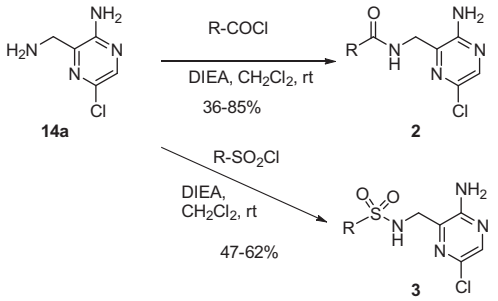
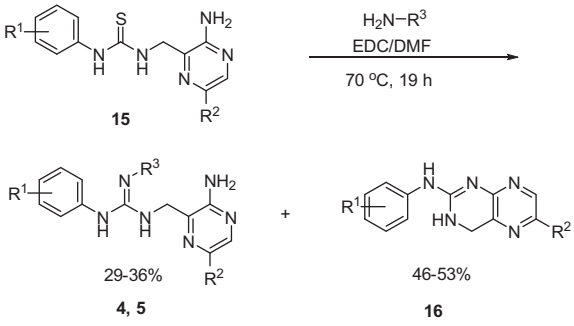


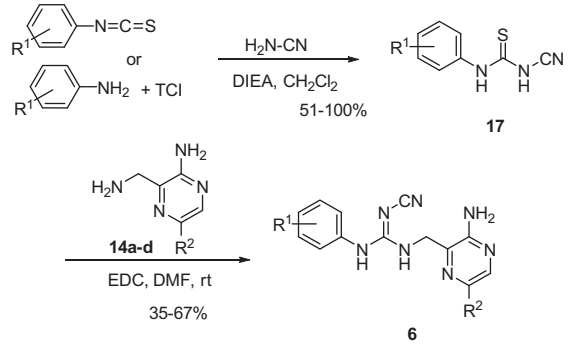
Figure 1. Structures of thiourea MK-2 inhibitor **1a** and designed non-thiourea aminopyrazines **1b**, **2**–**13**.



Scheme 1. Synthesis of amides and sulfonamides **2** and **3**.



Scheme 2. Synthesis of *N*-alkyl and *N*-alkoxy guanidines **4** and **5**.



Scheme 3. Synthesis of *N*-cyano guanidines **6**.

Cyanothioureas **17** were prepared in good to high yields from treatment of cyanoamine with corresponding anilines in the presence of 1,1′-thiocarbonyldiimidazole (TCI) and DIEA, or with corresponding isothiocyanates in the presence of DIEA. Reaction of thioureas **17** with (5-substituted-2-aminopyrazin-3-yl)methyl amines **14a–d** in the presence of EDC in DMF at room temperature afforded desired cyanoguanidines **6** in moderate to good yields.

To prepare *N*-acyl guanidines **7**, *N*-acyl thioureas **18** were made from the corresponding acyl isothiocyanates and anilines in the presence of DIEA (Scheme 4). Treatment of **18** with (5-substituted-2-aminopyrazin-3-yl)methyl amines **14** in the presence of EDC in DMF at room temperature afforded desired cyanoguanidines **7** in moderate to good yields. Similarly, *N*-sulfonyl guanidines **8** was synthesized from corresponding *N*-sulfonyl thiourea **19** with (5-cyclopropyl-2-aminopyrazin-3-yl)methyl amine **14c** in good yield (Scheme 5).

2-Aminobenzothiazole **9a** and 2-aminoquinolines **9b–d** were prepared by treating (2-chloro-5-aminopyrazin-6-yl)methyl amine **14a** with corresponding 2-chlorobenzothiazole or 2-chloroquinolines. The reactions were carried out in the presence of K_2CO_3 at 160 °C in a microwave reactor for 10–25 min (Scheme 6).