# Design, Synthesis, and Structure−Activity Relationship of Substrate Competitive, Selective, and in Vivo Active Triazole and Thiadiazole Inhibitors of the c-Jun N-Terminal Kinase

Surya K. De,[†] John L. Stebbins,[†] Li-Hsing Chen,[†] Megan Riel-Mehan,[†] Thomas Machleidt,[‡] Russell Dahl,[†] Hongbin Yuan,[†] Aras Emdadi,[†] Elisa Barile,[†] Vida Chen,[†] Ria Murphy,[†] and Maurizio Pellecchia*,[†]

*Infectious and Inflammatory Disease Center and Cancer Center, Burnham Institute for Medical Research, 10901 North Torrey Pines Road, La Jolla, California 92037, and Invitrogen Discovery Services, Invitrogen Corporation, 501 Charmany Drive, Madison, Wisconsin 53719*

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We report comprehensive structure−activity relationship studies on a novel series of c-Jun N-terminal kinase (JNK) inhibitors. The compounds are substrate competitive inhibitors that bind to the docking site of the kinase. The reported medicinal chemistry and structure-based optimizations studies resulted in the discovery of selective and potent thiadiazole JNK inhibitors that display promising in vivo activity in mouse models of insulin insensitivity.

## Introduction

Protein kinases comprise 2% of the mammalian genome and catalyze the transfer of $\gamma$-phosphoryl group of ATP to specific protein substrates.[1] The c-Jun N-terminal kinases (JNKs[a]) are a series of serine/threonine protein kinases of the mitogen activated protein kinase (MAPK) family. Three distinct genes encoding JNKs have been identified, JNK-1, JNK-2, and JNK-3, and at least 10 different splicing isoforms exist in mammalian cells.[2−4] The three JNK isoforms share more than 90% amino acid sequence identity, and the ATP pocket is >98% homologous. These proteins are activated in response to cellular stresses such as heat shock, irradiation, hypoxia, chemotoxins, and peroxides. They are also activated in response to various cytokines and participate in the onset of apoptosis.[5,6] It is reported that up-regulation of JNK activity is associated with a number of disease states such as type-2 diabetes, obesity, cancer, inflammation, and stroke.[1−3] Therefore, JNK inhibitors are expected to be effective therapeutic agents against a variety of diseases.

JNKs bind to substrates and scaffold proteins, such as JIP-1, that contain a D-domain, as defined by the consensus sequence R/KXXXXLXL.[7,8] A peptide corresponding to the D-domain of JIP-1 (aa 153−163; pep-JIP1) inhibits JNK activity in vitro and displays remarkable selectivity with little inhibition of the closely related Erk and p38 MAPKs.[9−12] Recent in vivo data, generated for studies focusing on pep-JIP1 fused to the cell permeable HIV-TAT peptide, show that its administration in various mouse models of insulin resistance and type-2 diabetes restores normoglycemia without causing hypoglycemia in lean mice.[13] The peptide was further improved by the synthesis of an all-D retroinverso peptide, D-JNK1, containing a cell-penetrating sequence. However, the peptide's instability in vivo, its short half-life, and the costly and inefficient large-scale manufacturing and purification processes all hamper the development of novel therapies for diabetes based on D-JNK1 alone. Nevertheless, despite some skepticism surrounding this peptide, the efficacy of such substrate competitive peptides is such that several recent studies report preliminary success on the use of D-JNK1 in early preclinical and clinical studies.[9−13]

There has been considerable effort to identify JNK inhibitors over the past several years.[14−20] A drug discovery program in our laboratory was initiated with the aim of identifying and characterizing small-molecule JNK inhibitors as novel chemical entities targeting the JIP docking site.[21,22] As a result of these efforts, we have reported on the identification of compound **12** (BI-78D3) (Figure 1A) (4-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol) from our HTS libraries as the first potent and selective JNK inhibitor of this class with demonstrated in vivo activity in mice model of insulin resistance.[21]

As a continuation of our work[21,22] we now report a comprehensive structure−activity relationship studies describing the discovery of novel JNK inhibitors that target the JIP-JNK interaction site. We developed a triazole series followed by a thiadiazole series based on structure−activity relationship (SAR) studies carried out on the initial hit compound **12** (Figure 1A)[21] which ultimately led to the discovery of compound **9** (Figure 1B). We describe here the pharmacological properties, design, and SAR studies that have led to its identification.

## Results and Discussion

Screening of our internal compound collection for JNK inhibitors resulted in the identification of compounds belonging to the triazole series.[21] The 4-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (compound **12**, Figure 1A)[21] was qualified as a hit and became the starting point of our medicinal chemistry efforts.

To investigate the effects on potency induced by small changes in the structure of **12**, we developed the general synthetic route for the preparation of this series. Compound **1**, thiobiurea, was synthesized according to the reported procedure (Scheme 1).[23] A variety of commercially available aryl isothiocyanates were treated with semicarbazide in the presence of sodium acetate in acetonitrile at room temperature to give **1a**−**q** and **4a**−**e** in good yields (Scheme 1). The thiobiureas (**1a**−**e**) and **4a**−**e** were heated at 100 °C in the presence of 2 M NaOH aqueous solution for 5−8 h to afford 3-hydroxy-5-thiol derivatives of triazoles in moderate to good yields. Compounds **3a**−**q** were synthesized by nucleophilic substitution of 2-bromo-5-nitrothiazole with thiol of triazoles in NaOMe/MeOH solution.

* To whom correspondence should be addressed. Phone: 858-646-3159. Fax: 858-713-9925. E-mail: mpellecchia@burnham.org.

[†] Burnham Institute for Medical Research.

[‡] Invitrogen Corporation.

[a] Abbreviations: JNK, c-Jun N-terminal protein kianse; MAPK, mitogen-activated protein kinase; JIP1, JNK-interacting protein 1; EDC, *N*-ethyl-*N*′-(3-dimethylaminopropyl)corbodiimide; DMF, *N*,*N*-dimethylformamide.

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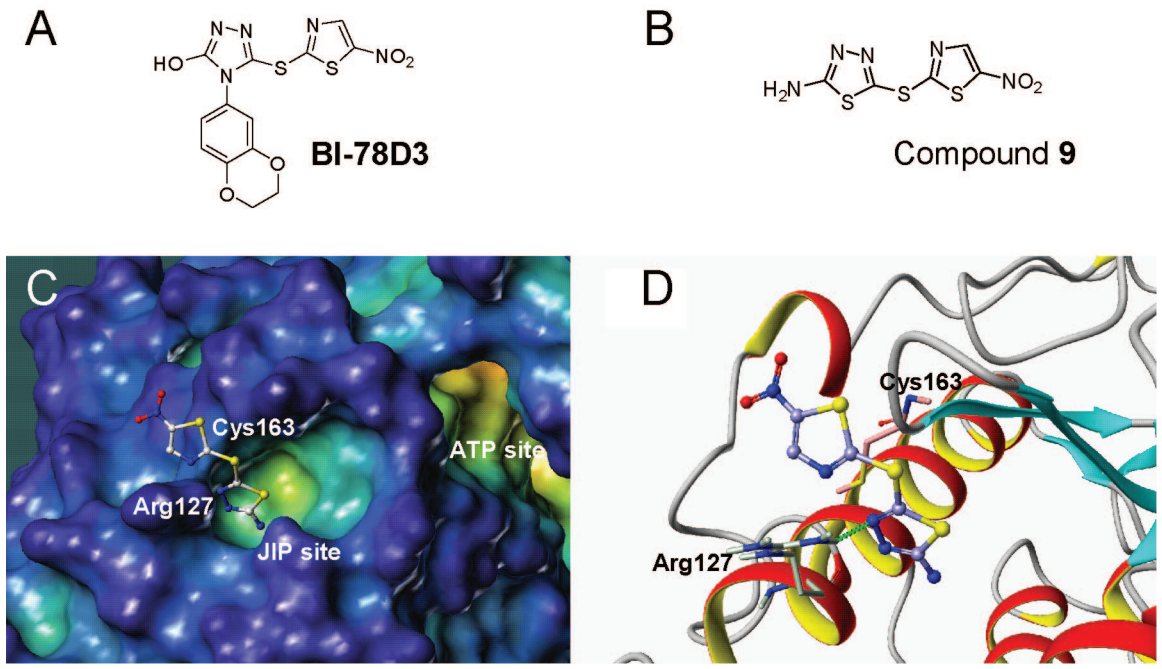
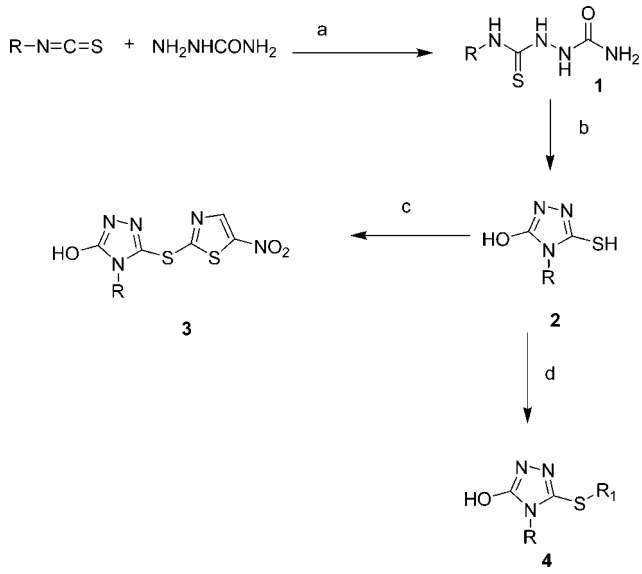



**Figure 1.** Chemical structures and docked geometry: (A) chemical structure of the previously reported compound **12**;[21] (B) chemical structure of compound **9**; (C, D) docked structure of compound **9** in the JIP site of JNK1.

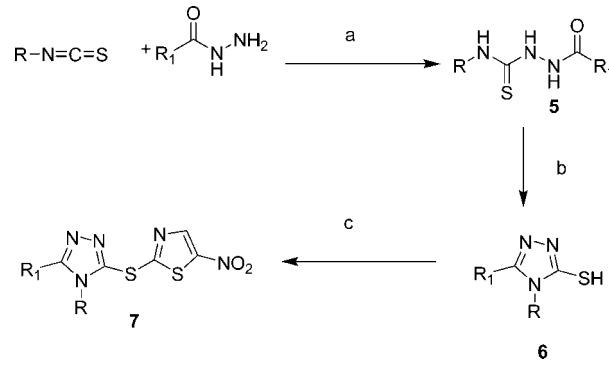
**Scheme 1**[a]




[a] Reagents and conditions: (a) sodium acetate, $CH_3CN$, room temp; (b) 2 M NaOH solution, 100 °C; (c) sodium methoxide, MeOH, 2-bromo-5-nitrothiazole, room temp; (d) aryl or alkyl bromide, sodium methoxide, MeOH, room temp. **1**−**3**: (**a**) R = 4-*tert*-butylphenyl; (**b**) R = 3,4-methylenedioxyphenyl; (**c**) R = 4-fluorophenyl; (**d**) R = 2,4-dimethoxyphenyl; (**e**) R = 3,5-dimethoxyphenyl; (**f**) R= 3,4-dimethoxyphenyl; (**g**) R = naphthyl; (**h**) R = 2,5-dimethoxyphenyl; (**i**) R = 4-trifluromethoxyphenyl; (**j**) R = 4-nitrophenyl; (**k**) R = 3,4-difluorophenyl; (**l**) R = 3-fluorophenyl; (**m**) R = 3-methoxyphenyl,; (**n**) R = 2-methoxyphenyl; (**o**) R = 3-trifluoromethylphenyl; (**p**) R = 4-trifluromethylphenyl; (**q**) R = cyclohexyl; (**r**) R = 4-methoxyphenyl. **4a**: R = phenyl, $R_1$ = *n*-butyl. **4b**: R = phenyl, $R_1$ = ethyl. **4c**: R = 3,4-benzodioxine, $R_1$ = benzyl. **4d**: R= phenyl, $R_1$ = 5-nitrothiophene.

We found that the thiol is consistently more reactive than hydroxyl; we never isolated any hydroxyl derivative compounds, even using excess amounts of 2-bromo-5-nitrothiazole. Similarly, the compounds **4a**−**e** were prepared using different alkyl and aryl bromides.

**Scheme 2**[a]

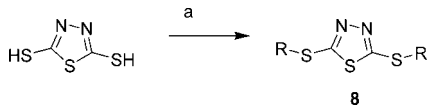



[a] Reagents and conditions: (a) sodium acetate, $CH_3CN$, room temp; (b) 2 M NaOH solution, 100 °C; (c) sodium methoxide, MeOH, 2-bromo-5-nitrothiazole, room temp. **5-7**: (**a**) R = 3,4-benzodioxine, $R_1$ = phenyl; (**b**) R = 2,4-dichlorophenyl, $R_1$= 1-methylpyrole; (**c**) R = 3-methoxyphenyl, $R_1$ = phenyl; (**d**) R = furfuryl; $R_1$ = naphthyl; (**e**) R = phenyl, $R_1$ = thiophene; (**f**) R = phenyl, $R_1$ = 4-pyridyl; (**g**) R = methyl, $R_1$ = pyrazinyloxadiazole.

Exploration of triazole C-3 position (replacing hydroxyl to alky or aryl) required a different synthesis of the core scaffold. Arylbithioureas (**5a**−**g**) (Scheme 2) were prepared according to published procedures.[24] Compounds **6a**−**g** were synthesized following the similar conditions mentioned before in Scheme 1, and a few of them were commercially available (**6b**,**d**−**g**). The final compounds (**7a**−**g**) were successfully obtained by displacing bromide with thiols as previously described. The dithioether of thiadiazoles (**8a**−**g**) were synthesized from commercially available 1,3,4-thiadiazole-2,5-dithiol (Scheme 3). Similarly, we prepared compounds **9** and **10** from 5-amino-1,3,4-thiadiazole-2-thiol to introduce further diversity into the series (Scheme 4).

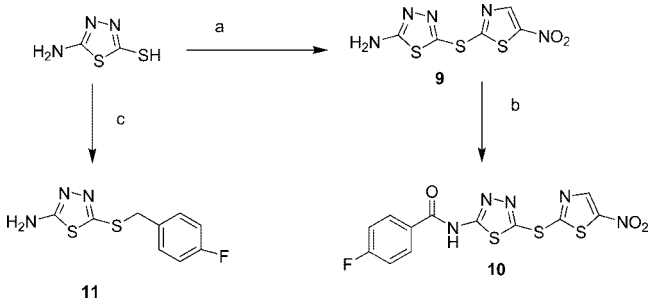
In our work, the basis for the biochemical assay was JNK-1 mediated phosphorylation of ATF-2. High-throughput screening identified compound **12**, with an $IC_{50}$ of 280 nM.[21] Competition experiments confirmed that this compound is a competitive inhibitor of the interactions between JNK and pepJIP1.[21]

**Scheme 3**[a]



[a] Reagents and conditions: (a) alkyl or aryl bromide, sodium methoxide, MeOH, room temp. **8a**: R = benzyl. **8b**: R = 4-fluorobenznyl. **8c**: R = 4-methoxybenzyl. **8d**: R = 3-nitrobenzyl. **8e**: R = 4-nitrobenzyl. **8f**: R = 5-nitrothiazole. **8g**: R = 5-carboxylthiazole.

**Scheme 4**[a]



[a] Reagents and conditions: (a) 2-bromo-5-nitrothiazole, sodium methoxide, MeOH, room temp; (b) 4-fluorobenzoic acid, EDC, HOBt, DIEA, DMF, 50 °C; (c) 4-fluorobenzyl bromide, sodium methoxide, MeOH, room temp.

**Table 1.** Triazole Series

| compd | LanthaScreen kinase activity assay, $IC_{50}$ ($\mu$M) or % inhibition at 100 $\mu$M | pepJIP1 DELFIA displacement assay, $IC_{50}$ (nM) or % inhibition at 100 $\mu$M |
|---|---|---|
| **4a** | 25% | ND[a] |
| **4b** | 16% | ND[a] |
| **4c** | 2% | ND[a] |
| **4d** | 22% | 55% |
| **3a** | 39 | 95 |
| **3b** | 3.3 | 102 |
| **3c** | 1.8 | 225 |
| **3d** | 1.1 | 66 |
| **3e** | 4.3 | 105 |
| **3f** | 1.0 | 43 |
| **3g** | 2.7 | 48 |
| **3h** | 2.4 | 85 |
| **3i** | 2 | 130 |
| **3j** | 0.4 | 86 |
| **3k** | 6.6 | 73% |
| **3l** | 84 | 626 |
| **3m** | 16 | 118 |
| **3n** | 2.2 | 53 |
| **3o** | 2.9 | 160 |
| **3p** | 3 | 75 |
| **3q** | 25 | 56 |
| **3r** | 17 | 45 |

[a] ND: no displacement at 100 $\mu$M.

We began our investigation of this initial scaffold by synthesizing compounds with variations in the 3-, 4-, and 5-positions of the triazol. As shown in Table 1, among the combinations tested, changes in the 4- and 5-positions are well tolerated. We first synthesized and tested a series of alkyl thio ethers, but none of these showed any remarkable JNK inhibition (**4a**, **4b** in Table 1). Aryl and benzyl thio ethers lead to a drastic drop in activity. Replacing the thiazole with a thiophene also results in a decrease in activity. We found that the only suitable moiety in the 3-position was a 5-nitrothiazole, and changing from nitro to carboxyl group on the thiazole unit was also not tolerated. On the basis of our results, we retained the 5-position as a 5-nitrothiazole and varied the other positions of the parent compound **12** (Figure 1A).

**Table 2.** Modification of C-3 Position (Replacing Hydroxyl with Alkyl or Aryl) on Triazoles

| compd | LanthaScreen kinase activity assay, $IC_{50}$ ($\mu$M) | pepJIP1 DELFIA displacement assay, $IC_{50}$ (nM) |
|---|---|---|
| **7a** | 27 | 108 |
| **7b** | 2 | 83 |
| **7c** | 7.9 | 168 |
| **7d** | 13.6 | 57 |
| **7e** | >50 | 107 |
| **7f** | 3.1 | 122 |
| **7g** | 100 | 131 |

**Table 3.** Dithioether of 1,3,4-Thiadiazoles

| compd | LanthaScreen kinase activity assay, $IC_{50}$ ($\mu$M) | pepJIP1 DELFIA displacement assay, $IC_{50}$ (nM) |
|---|---|---|
| **8a** | >100 | ND[a] |
| **8b** | >100 | ND[a] |
| **8c** | >100 | ND[a] |
| **8d** | >100 | ND[a] |
| **8e** | >100 | ND[a] |
| **8f** | 0.8 | 187 |
| **8g** | 84 | 100 |

[a] ND: no displacement at 100 $\mu$M.

Modifications investigated in the 4-phenyl moiety of the triazole series are detrimental to JNK potency as shown in Table 1. The 3- and 4-positions of the phenyl group were more active. Compound **3f** has a 3,4-methylenedioxyphenyl group in place of the 3,4-benzodioxin ring in the parent compound and has comparable activity. However, a 4-*tert*-butylphenyl group was not tolerated in this position likely because of its bulky size (**3a**). The most active compound contains 4-nitrophenyl on the 4-position of the triazole, resulting in an $IC_{50}$ of 0.4 $\mu$M (**3j**); likewise, groups such as fluoro (**3c**), methoxy (**3d**, **3e**, **3f**, **3h**, **3m**, **3n**, and **3r**), trifluoromethyl (**3o** and **3p**), trifluoromethoxy (**3i**) show similar activities. However, a 3-fluoro on phenyl group (**3l**) does not show any good activity and 3,4-difluorophenyl (**3k**) has a moderate activity.

We further modified the 4- and 5-positions on the triazole ring while maintaining the 5-nitrothiazole in the 3-position (Table 2). In this series, phenyl group in the 5-position reduces the activity in the kinase assay compared to the parent compound **12** but has a lower $IC_{50}$ (108 nM) in the displacement assay against pepJIP1, whereas replacing the dioxin ring with a 3-methoxypenyl group (**7c**) results in a 3-fold increase in activity, indicating that two large ring structures in both the 4- and 5-positions may constitute too much bulk. We subsequently synthesized compounds containing smaller aromatic structures in both positions (**7b** and **7d)** and found that 1-methylpyrrole is tolerated while both naphthyl and pyrazinyloxadiazole in the 5-position reduce the activity. We next focused on a 1,3,4-thiadiazole-2,5-dithiol as the scaffold ring to further explore the effect of the substituents in the 5-position (Table 3). We had very little success with benzyl groups, but smaller moieties such as a nitrothiazole and a carboxylthiazole were very promising, which led to compound **9** (Table 4). The use of 5-amino-1,3,4-thiadiazole-2-thiol as a scaffold allowed for nonsymmetrical modifications; however, further modifications of the amine group on compound **9** did not increase potency (**10**). Addition of a methylphenyl group on the thiol resulted in a complete loss of activity (**11**).

Modeling studies suggest that compound **9** may bind at the JIP site with the nitrothiazol group crossing the ridge close to residues Arg127 and Cys163 (Figure 1C and Figure 1D). Its thiazole group can form a hydrogen bond with Arg127 and can

**Table 4.** 2-Amino-1,3,4-thiadiazole Series

| compd | LanthaScreen kinase activity assay, $IC_{50}$ ($\mu$M) | pepJIP1 DELFIA displacement assay, $IC_{50}$ (nM) |
|---|---|---|
| **9** | 0.7 | 239 |
| **10** | 84 | ND[a] |
| **11** | >100 | >1000 |

[a] ND: no displacement at 100 $\mu$M.

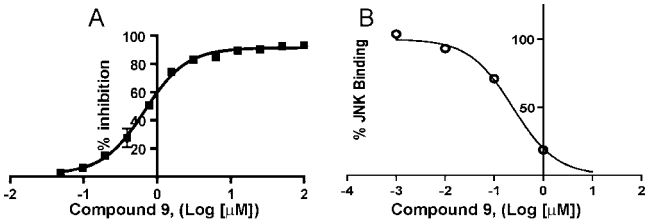



**Figure 2.** In vitro assays: (A) kinase inhibition assay for **9**; (B) dose dependent displacement of biotinylated pepJIP1 from GST-JNK1.

**Table 5.** Selectivity Profile

| compd | p38α, $IC_{50}$ ($\mu$M) | Akt1, $IC_{50}$ | furin, $IC_{50}$ ($\mu$M) | LF, $IC_{50}$ ($\mu$M) |
|---|---|---|---|---|
| **3j** | >100 | 20% at 100 $\mu$M | >50 | >100 |
| **7b** | >100 | 14% at 100 $\mu$M | >50 | >100 |
| **8f** | >100 | 27% at 100 $\mu$M | >50 | >100 |
| **9** | >100 | 11% at 100 $\mu$M | >50 | >100 |

bind into a subpocket nearby, which on the basis of this hypothesis could tolerate groups with a similar size to substitute the thiadiazole group of compound **9**.

Compound **9** showed an $IC_{50}$ of 0.7 $\mu$M in the kinase assay (Figure 2A) in a substrate competitive manner. In addition, compound **9** also showed an $IC_{50}$ of 239 nM in displacing pepJIP1, as measured by a DELFIA assay (Figure 2B).[21] Compound **9** was found to be 142-fold less active against p38α, a member of the MAPK family with high structural similarity to JNK and completely inactive against Akt kinase (Akt inhibitions at 100 $\mu$M for compounds **3j**, **7b**, **8f**, and **9** were 20%, 14%, 27%, and 11%, respectively). These compounds were also very selective against other protein targets under investigation in our laboratory including the metalloprotease LF (lethal factor) and the proprotein convertase furin (Table 5). This selectivity is in agreement with our previous findings with compound **12** and with the reported data on pepJIP1.[10−13,21]

In an attempt to profile the properties of compound **9** in the context of a complex cellular milieu, we employed the cell-based LanthaScreen kinase assay. In this assay compound **9** is able to inhibit TNF-α stimulated phosphorylation of c-Jun in cell ($EC_{50}$ = 6.23 $\mu$M; Figure 3A). It is noted that the cell-based system employed makes use of a GFP-c-Jun stable expression system. As a result, the levels of GFP-c-Jun in these cells are higher than endogenous levels. This could have an inflationary effect on the $EC_{50}$ values obtained with this assay when testing substrate competitive compounds. Nonetheless, this finding establishes that compound **9** is able to function in a cellular context.

The link between the JNK pathway and type-2 diabetes has been established previously.[10−13] Thus, in an attempt to further our bioanalysis of the JNK-inhibitory properties of compound **9**, we monitored the ability of compound **9** to restore insulin sensitivity in a mouse model of type-2 diabetes. For this analysis, insulin insensitive mice from Harlan (Harlan Sprague Dawley, Inc.; Indianapolis, IN) were injected once with 25 mg/kg of compounds **9**, **7b**, and **8f** 30 min prior to insulin injection. The effect of insulin on blood glucose levels was then measured (Figure 3B). Compound **9** resulted in a statistically significant reduction in blood glucose levels compared to the vehicle control (Figure 3B). Hence, the ability of compound **9** to restore insulin sensitivity is consistent with its proposed function as an effective JNK inhibitor.[21] Liquid chromatography/mass spectrometry bioavailability analysis demonstrates that compound **9** has favorable microsomal and plasma stability ($T_{1/2}$ = 27 min; see Supporting Information) which support its use in further in vivo experiments.

## Conclusion

We successfully developed a new series of JNK inhibitors, many of which are very potent in vitro. Our initial in vivo screens indicate that compound **9** possesses the ability to restore insulin sensitivity in mice models of diabetes. Our results indicate that targeting the protein−protein interaction between JNK and JIP with a small molecule is a new and promising avenue for the development of novel pharmacological tools that inactivate the JNK pathway.

## Experimental Section

**General.** Unless otherwise indicated, all anhydrous solvents were commercially obtained and stored in SureSeal bottles under nitrogen. All other reagents and solvents were purchased as the highest grade available and used without further purification. Thin-layer chromatography (TLC) analysis of reaction mixtures was performed using Merck silica gel 60 F254 TLC plates and visualized using ultraviolet light. NMR spectra were recorded on a Varian 300 or 500 MHz instrument. Chemical shifts ($\delta$) are reported in parts per million (ppm) referenced to $^1$H ($Me_4Si$ at 0.00). Coupling constants ($J$) are reported in Hz throughout. Mass spectral data were acquired on Shimadzu LCMS-2010EV for low resolution and on an Agilent ESI-TOF for either high or low resolution. Purity of all compounds was obtained in a HPLC Breeze from Waters Co. using an Atlantis T3 3 $\mu$m, 4.6 mm × 150 mm reverse phase column. The eluant was a linear gradient with a flow rate of 1 mL/min from 95% A and 5% B to 5% A and 95% B in 15 min followed by 5 min at 100% B (solvent A, $H_2O$ with 0.1% TFA; solvent B, ACN with 0.1% TFA). The compounds were detected at $\lambda$ = 254 nm. Purity of key compounds was established by elemental analysis as performed on a Perkin-Elmer series II-2400 (see Supporting Information).

**2-(4-*tert*-Butylphenylcarbamothioyl)hydrazinecarboxamide (1a).** To a suspension of sodium acetate (216 mg, 2.63 mmol) and semicarbazide (293 mg, 2.63 mmol) in acetonitrile (10 mL) was added 4-*tert*-butylphenyl isothiocyanate (506 mg, 2.63 mmol), and the reaction mixture was stirred for 24 h. The solvent was removed by rotary evaporation, and the resulting residue was dissolved in 2 M NaOH aqueous solution (30 mL) and filtered through activated charcoal to remove any unreacted isothiocyanate. The filtrate was acidified with 1 N HCl (40 mL). The precipitate was collected and washed with water (3 × 20 mL) and hexanes (2 × 20 mL) to afford **1a** as a white solid (601 mg, 86%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.26 (s, 9 H), 6.03 (s, 2 H, $NH_2$), 7.31 (d, $J$ = 8.4 Hz, 2 H), 7.39 (d, $J$ = 8.1 Hz, 2 H), 7.99 (s, 1 H, NH), 9.32 (s, 1 H), NH), 9.55 (s, 1 H, NH); MS $m/z$ 555(2M + Na)$^+$, 533 (2M + H)$^+$, 289 (M + Na)$^+$, 267 (M + H)$^+$, 250, 224, 158, 88, 74; HRMS calcd for $C_{12}H_{19}N_4OS$ (M + H) 267.1274, found 267.1279.

Following the above-mentioned procedure and with the appropriate starting materials and reagents used, compounds **1b**−**q** were synthesized.

**2-(Benzo[*d*][1,3]dioxol-5-ylcarbamothioyl)hydrazinecarboxamide (1b).** Yield, 88%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 5.99 (s, 2 H), 6.03 (s, 2 H, $NH_2$), 6.80−6.86 (m, 2 H), 7.09 (s, 1 H), 7.97 (s, 1 H, NH), 9.32 (s, 1 H, NH), 9.54 (s, 1 H, NH); MS $m/z$ 531 (2M + Na)$^+$, 277 (M + Na)$^+$, 255 (M + H)$^+$, 238, 212, 151,

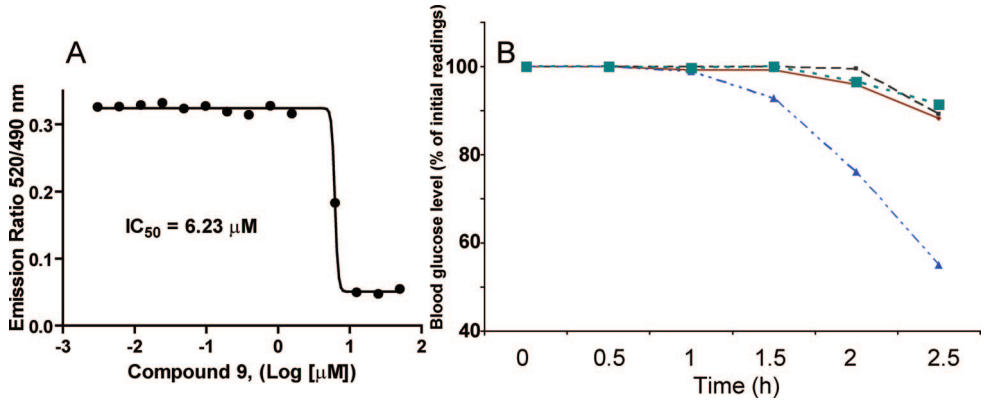



**Figure 3.** Cell based and in vivo efficacy studies with compounds **9** and **7b**: (A) TR-FRET analysis of c-Jun phosphorylation upon TNF-α stimulation of HeLa cells in the presence of increasing concentrations **9**; (B) effects on insulin resistance in 11-week-old BKS.Cg−+Lepr$^{db}$/+Lepr$^{db}$/OlaHsd db/db mice (Harlan Sprague Dawley, Inc.; Indianapolis, IN); (diamonds) vehicle control; (triangles) 25 mg/kg **9**; (circles) 25 mg/kg **7b**; (squares) 25 mg/kg **8f**. Data shown are the mean values ± SD ($n = 6$): (*) $P = 0.0022$; (**) $P = 0.0001$.

147, 129, 106, 102, 97; HRMS calcd for $C_9H_{11}N_4O_3S$ (M + H) 255.0546, found 255.0546.

**2-(4-Fluorophenylcarbamothioyl)hydrazinecarboxamide (1c).** Yield, 76%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.05 (s, 2 H, $NH_2$), 7.10−7.18 (m, 2 H), 7.44−7.50 (m, 2 H), 8.02 (s, 1 H, NH), 9.41 (s, 1 H, NH), 9.69 (s, 1 H, NH); MS *m/z* 251 (M + Na)$^+$, 229 (M + H)$^+$, 212, 192, 186, 141, 129, 106, 102, 97, 85; HRMS calcd for $C_8H_{10}FN_4OS$ (M + H) 229.0554, found 229.0560.

**2-(2,4-Dimethoxyphenylcarbamothioyl)hydrazinecarboxamide (1d).** Yield, 81%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.74 (s, 3 H, OMe), 3.77 (s, 3 H, OMe), 6.07(s, 2 H, $NH_2$), 6.48 (d, $J = 8.1$ Hz, 1 H), 6.59 (s, 1 H), 7.84 (d, $J = 8.1$ Hz, 1 H), 8.08 (s, 1 H, NH), 8.93 (s, 1 H, NH), 9.36 (s, 1 H, NH); HRMS calcd for $C_{10}H_{15}N_4O_3S$ (M + H) 271.0865, found 271.0866.

**2-(3,5-Dimethoxyphenylcarbamothioyl)hydrazinecarboxamide (1e).** Yield, 85%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.06 (s, 2 H, $NH_2$), 6.26 (s, 1 H), 6.87 (s, 2 H), 7.99 (s, 1 H, NH), 9.40 (s, 1 H, NH), 9.56 (s, 1 H, NH); HRMS calcd for $C_{10}H_{15}N_4O_3S$ (M + H) 271.0865, found 271.0868.

**2-(3,4-Dimethoxyphenylcarbamothioyl)hydrazinecarboxamide (1f).** Yield, 82%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.04 (s, 2 H, $NH_2$), 6.86 (d, $J = 8.7$ Hz, 1 H), 6.99 (d, $J = 8.7$ Hz, 1 H), 7.25 (s, 1 H), 7.99 (s, 1 H, NH), 9.28 (s, 1 H, NH), 9.51 (s, 1 H, NH); HRMS calcd for $C_{10}H_{15}N_4O_3S$ (M + H) 271.0865, found 271.0864.

**2-(Naphthalen-1-ylcarbamothioyl)hydrazinecarboxamide (1g).** Yield, 79%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.10 (s, 2 H, $NH_2$), 7.38−7.55 (m, 4 H), 7.84 (d, $J = 7.8$ Hz, 1 H), 7.91−7.97 (m, 2 H), 8.21 (s, 1 H, NH), 9.46 (s, 1 H, NH), 9.86 (s, 1 H, NH); MS *m/z* 543 (2M + Na)$^+$, 521 (2M + H)$^+$, 283 (M + Na)$^+$, 261 (M + H)$^+$, 244, 218, 159, 144, 85, 74, 56; HRMS calcd $C_{12}H_{13}N_4OS$ (M + H) 261.0805, found 261.0809.

**2-(2,5-Dimethoxyphenylcarbamothioyl)hydrazinecarboxamide (1h).** Yield, 84%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.68 (s, 3 H, OMe), 3.77 (s, 3 H, OMe), 6.19 (s, 2 H, NH2), 6.65 (dd, $J = 9$ and 2.7 Hz, 1 H), 6.95 (d, $J = 9$ Hz, 1 H), 8.20 (s, 1 H), 8.28 (s, 1 H, NH), 9.14 (s, 1 H, NH), 9.62 (s, 1 H, NH); HRMS calcd for $C_{10}H_{15}N_4O_3S$ (M + H) 271.0865, found 271.0862.

**2-(4-(Trifluoromethoxy)phenylcarbamothioyl)hydrazinecarboxamide (1i).** Yield, 82%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.07 (s, 2 H, $NH_2$), 7.29 (d, $J = 8.4$ Hz, 2 H), 7.62 (d, $J = 9$ Hz, 2 H), 8.07 (s, 1 H, NH), 9.51 (s, 1 H, NH), 9.83 (s, 1 H, NH); HRMS calcd for $C_9H_{10}F_3N_4O_2S$ (M + H) 295.0477, found 295.047 78.

**2-(4-Nitrophenylcarbamothioyl)hydrazinecarboxamide (1j).** Yield, 88%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.13 (s, 2 H, $NH_2$), 7.96 (d, $J = 9.0$ Hz, 2 H), 8.13 (s, 1 H, NH), 8.18 (d, $J = 9.3$ Hz, 2 H), 9.80 (s, 1 H, NH), 10.13 (s, 1 H, NH); MS *m/z* 278 (M + Na)$^+$, 256 (M + H)$^+$, 253, 239, 169, 146, 107, 104, 87, 76; HRMS calcd for $C_8H_9N_5O_3S$ 256.0499 (M + H), found 256.0500.

**2-(3,4-Difluorophenylcarbamothioyl)hydrazinecarboxamide (1k).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.08 (s, 2 H, $NH_2$), 7.30−7.39 (m, 2 H), 7.70−7.724 (m, 1 H), 8.03 (s, 1 H, NH), 9.55 (s, 1 H, NH), 9.81 (s, 1 H, NH); MS *m/z* 515 (2M + Na)$^+$, 269 (M + Na)$^+$, 247 (M + H)$^+$, 230, 204, 192, 147, 121, 102, 97; HRMS calcd for $C_8H_9F_2N_4OS$ (M + H) 247.0460, found 247.0455.

**2-(3-Fluorophenylcarbamothioyl)hydrazinecarboxamide (1l).** Yield, 81%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.08 (s, 2 H, $NH_2$), 6.94 (t, $J = 8.4$ Hz, 1 H), 7.31−7.36 (m, 2 H), 7.58−7.63 (m, 1 H), 8.03 (s, 1 H, NH), 9.53 (s, 1 H, NH), 9.78 (s, 1 H, NH); MS *m/z* 251 (M + Na)$^+$, 229 (M + H)$^+$, 212, 186, 147, 118, 106, 85; HRMS calcd for $C_8H_{10}FN_4OS$ (M + H) 229.0554, found 229.0553.

**2-(3-Methoxyphenylcarbamothioyl)hydrazinecarboxamide (1m).** Yield, 84%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.73 (s, 3 H, OMe), 6.05 (s, 2 H, $NH_2$), 6.69 (d, $J = 7.8$ Hz, 1 H), 7.10 (d, $J = 7.8$ Hz, 1 H), 7.20 (t, $J = 7.8$ Hz, 1 H), 7.26 (s, 1 H), 8.01 (s, 1 H, NH), 9.40 (s, 1 H, NH), 9.61 (s, 1 H, NH); MS *m/z* 503 (2M + Na)$^+$, 481 (2M + H)$^+$, 263 (M + Na)$^+$, 241 (M + H)$^+$, 224, 198, 166, 147, 129, 118, 107, 97; HRMS calcd for $C_9H_{12}N_4O_2S$ (M + H) 241.0754, found 241.0755.

**2-(2-Methoxyphenylcarbamothioyl)hydrazinecarboxamide (1n).** Yield, 86%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.81 (s, 3 H, OMe), 6.15 (s, 2 H, $NH_2$), 6.94 (t, $J = 6.9$ Hz, 1 H), 7.04 (d, $J = 7.2$ Hz, 1 H), 7.09−7.14 (m, 2 H), 8.33 (s, 1 H, NH), 9.11 (1 H, NH), 9.56 (s, 1 H, NH); HRMS calcd for $C_9H_{12}N_4O_2S$ (M + H) 241.0754, found 241.0757.

**2-(3-(Trifluoromethyl)phenylcarbamothioyl)hydrazinecarboxamide (1o).** Yield, 69%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.10 (s, 2 H), 7.45−7.54 (m, 2 H), 7.87 (d, $J = 6.6$ Hz, 1 H), 8.00 (s, 1 H), 8.07 (s, 1 H), 9.61 (s, 1 H), 9.96 (s, 1 H); MS *m/z* 579 (2M + Na)$^+$, 557 (2M + H)$^+$, 301 (M + Na)$^+$, 279 (M + H)$^+$, 262, 236, 169, 126, 88, 74; HRMS calcd for $C_9H_9F_3N_4OS$ 279.0522 (M + H), found 279.0526.

**2-(4-(Trifluoromethyl)phenylcarbamothioyl)hydrazinecarboxamide (1p).** Yield, 56%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.10 (s, 2 H), 7.66 (d, $J = 8.7$ Hz, 2 H), 7.82 (d, $J = 8.4$ Hz, 2 H), 8.07 (s, 1 H), 9.63 (s, 1 H), 9.56 (s, 1 H); HRMS calcd for $C_9H_9F_3N_4OS$ 279.0522 (M + H), found 279.0524.

**2-(4-Methoxyphenylcarbamothioyl)hydrazinecarboxamide (1r).** Yield, 89%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.77 (s, 3 H, OMe), 6.02 (s, 2 H, $NH_2$), 6.87 (d, $J = 9$ Hz, 2 H), 7.33 (d, $J = 9$ Hz, 2 H), 7.98 (s, 1 H, NH), 9.28 (s, 1 H, NH), 9.53 (s, 1 H, NH); HRMS calcd for $C_9H_{12}N_4O_2S$ (M + H) 241.0754, found 241.0756.

**4-(4-*tert*-Butylphenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2a).** 2-Thiobiurea compound **1a** (502 mg, 1.88 mmol) in 2 M NaOH aqueous solution (20 mL) was stirred at reflux for 6 h. The reaction mixture was allowed to cool to room temperature and then acidified with 1 N HCl. The resulting precipitate was collected by filtration and washed with water (3 × 20 mL), hexanes (2 × 20 mL), 1:1 hexanes/diethyl ether (20 mL) successively and dried in vacuo to afford **2a**

as a white solid (355 mg, 76%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.31 (s, 9 H), 7.28 (d, $J$ = 8.4 Hz, 2 H), 7.51 (d, $J$ = 8.4 Hz, 2 H); MS $m/z$ 521 (2M + H)$^+$, 497 (2M − H)$^-$, 272 (M + Na)$^+$, 250 (M + H)$^+$, 151, 147, 134, 106, 97, HRMS calcd for $C_{12}H_{16}N_3OS$ (M + H) 250.1009, found 250.1005.

Following the above-mentioned procedure and with the appropriate starting materials and reagents used, compounds **2b**−**q** were prepared.

**4-(Benzo[*d*][1,3]dioxol-5-yl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2b).** Yield, 71%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.10 (s, 2 H), 6.80−7.01 (m, 3 H); MS $m/z$ 497 (2M + Na)$^+$, 475 (2M + H)$^+$, 260 (M + Na)$^+$, 238 (M + H)$^+$, 158, 120, 88, 56; HRMS calcd for $C_9H_8N_3O_3S$ (M + H) 238.0281, found 238.0278.

**4-(4-Fluorophenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2c).** Yield, 68%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.28−7.37 (m, 2 H), 7.40−7.46 (m, 2 H); MS $m/z$ 445 (2M + Na)$^+$, 423 (2M + H)$^+$, 234 (M + Na)$^+$, 212 (M + H)$^+$, 158, 88, 56; HRMS calcd for $C_8H_7FN_3OS$ (M + H) 212.0288, found 212.0289.

**4-(2,4-Dimethoxyphenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2d).** Yield, 65%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.72 (s, 3 H, OMe), 3.81 (s, 3 H, OMe), 6.58 (d, $J$ = 8.7 Hz, 1 H), 6.69 (s, 1 H), 7.08 (d, $J$ = 8.7 Hz, 1 H); MS $m/z$ 529 (2M + Na)$^+$, 507 (2M + H)$^+$, 276 (M + Na)$^+$, 254 (M + H)$^+$, 212, 158, 146, 141, 97, 88, 74, 56; HRMS calcd for $C_{10}H_{12}N_3O_3S$ (M + H) 254.0595, found 254.0586.

**4-(3,5-Dimethoxyphenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2e).** Yield, 68%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.75 (s, 6 H, 2 × OMe), 6.54 (d, $J$ = 2.1 Hz, 2 H), 6.57 (d, $J$ = 2.4 Hz, 1 H); HRMS calcd for $C_{10}H_{12}N_3O_3S$ (M + H) 254.0595, found 254.0588.

**4-(3,4-Dimethoxyphenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2f).** Yield, 71%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.72 (s, 3 H, OMe), 3.79 (s, 3 H, OMe), 6.87 (d, $J$ = 8.4 Hz, 1 H), 6.94 (s, 1 H), 7.02 (d, $J$ = 8.4 Hz, 1 H); HRMS calcd for $C_{10}H_{12}N_3O_3S$ (M + H) 254.0595, found 254.0588.

**5-Mercapto-4-(naphthalen-1-yl)-4*H*-1,2,4-triazol-3-ol (2g).** Yield, 74%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.48−768 (m, 5 H), 8.01−8.112 (m, 2 H); MS $m/z$ 509 (2M + Na)$^+$, 487 (2M + H)$^+$, 266 (M + Na)$^+$, 244 (M + H)$^+$, 186, 158, 144, 116, 85, 74, 67, 56; HRMS $C_{12}H_{10}N_3OS$ (M + H) 244.0539, found 244.0530.

**4-(2,5-Dimethoxyphenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2h).** Yield, 68%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.68 (s, 3 H, OMe), 3.71 (s, 3 H, OMe), 6.83 (d, $J$ = 2.7 Hz, 1 H), 7.03 (dd, $J$ = 8.4 and 2.7 Hz, 1 H), 7.08 (d, $J$ = 8.4 Hz, 1 H); HRMS calcd for $C_{10}H_{12}N_3O_3S$ (M + H) 254.0595, found 254.0589.

**5-Mercapto-4-(4-(trifluoromethoxy)phenyl)-4*H*-1,2,4-triazol-3-ol (2i).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.45−7.58 (m, 4 H); MS $m/z$ 577 (2M + Na)$^+$, 300 (M + Na)$^+$, 278 (M + H)$^+$, 201, 178, 141, 134, 129, 106, 97, 85; HRMS calcd for $C_9H_7F_3N_3O_2S$ (M + H) 278.0206, found 278.0211.

**5-Mercapto-4-(4-nitrophenyl)-4*H*-1,2,4-triazol-3-ol (2j).** Yield, 25%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.79 (d, $J$ = 9.3 Hz, 2 H), 8.35 (d, $J$ = 9.0 Hz, 2 H); MS $m/z$ 261 (M + Na)$^+$, 242, 239 (M + H)$^+$, 229, 130, 88, 85; HRMS calcd for $C_8H_6N_4O_3S$ 239.0233 (M + H), found 239.0229.

**4-(3,4-Difluorophenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2k).** Yield, 57%; $^1$H NMR (300 MHz, $CD_3OD$) $\delta$ 7.02−7.08 (m, 1 H), 7.14−7.27 (m, 2 H); MS $m/z$ 252 (M + Na)$^+$, 230 (M + H)$^+$, 184, 169, 107, 95, 85, 56; HRMS calcd for $C_8H_6F_2N_3OS$ (M + H) 230.0194, found 230.0191.

**4-(3-Fluorophenyl)-5-mercapto-4*H*-1,2,4-triazol-3-ol (2l).** Yield, 56%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.24−7.35 (m, 3 H), 7.52−7.56 (m, 1 H); HRMS calcd for $C_8H_7FN_3OS$ (M + H) 212.0288, found 212.0286.

**5-Mercapto-4-(3-methoxyphenyl)-4*H*-1,2,4-triazol-3-ol (2m).** Yield, 74%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.94 (d, $J$ = 8.7 Hz, 1 H), 6.96 (s, 1 H), 7.02 (d, $J$ = 8.4 Hz, 1 H), 7.40 (t, $J$ = 7.5 Hz, 1 H); MS $m/z$ 246 (M + Na)$^+$, 224 (M + H)$^+$, 192, 147, 134, 106, 97; HRMS calcd for $C_9H_{10}N_3O_2S$ (M + H) 224.0488, found 224.0488.

**=5-Mercapto-4-(2-methoxyphenyl)-4*H*-1,2,4-triazol-3-ol (2n).** Yield, 65%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.74 (s, 3 H, OMe), 7.04 (t, $J$ = 7.2 Hz, 1 H), 7.15−7.24 (m, 2 H), 7.4 (t, $J$ = 7.2 Hz, 1 H); HRMS calcd for $C_9H_{10}N_3O_2S$ (M + H) 224.0488, found 224.0486.

**5-Mercapto-4-(3-(trifluoromethyl)phenyl)-4*H*-1,2,4-triazol-3-ol (2o).** Yield, 68%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.75−7.77 (m, 2 H), 7.82 (d, $J$ = 3.9 Hz, 1 H), 7.86 (s, 1 H); MS $m/z$ 543 (2M + Na)$^+$, 284 (M + Na)$^+$, 262 (M + H)$^+$, 226, 159, 141, 102, 74; HRMS calcd for $C_9H_9F_3N_4OS$ 279.0522 (M + H), found 279.0526.

**5-Mercapto-4-(4-(trifluoromethyl)phenyl)-4*H*-1,2,4-triazol-3-ol (2p).** Yield, 45%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.58 (d, $J$ = 9.0 Hz, 2H), 8.06 (d, $J$ = 8.4 Hz, 2H); MS $m/z$ 523 (2M + H)$^+$, 284 (M + Na)$^+$, 262 (M + H)$^+$, 260 (M − H)$^-$, 238, 102, 91, 74; HRMS calcd for $C_9H_6F_3N_3OS$ 262.0256 (M + H), found 262.0253.

**4-Cyclohexyl-5-mercapto-4*H*-1,2,4-triazol-3-ol (2q). 2q** was commercially available.

**5-Mercapto-4-(4-methoxyphenyl)-4*H*-1,2,4-triazol-3-ol (2r).** Yield, 69%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.79 (s, 3 H, OMe), 7.02 (d, $J$ = 7.5 Hz, 2 H), 7.25 (d, $J$ = 7.5 Hz, 2 H); HRMS calcd for $C_9H_{10}N_3O_2S$ (M + H) 224.0488, found 224.0489.

**4-(4-*tert*-Butylphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3a).** To a solution of **2a** (94 mg, 0.377 mmol) in MeOH (2 mL) was added MeONa (0.75 mL, 0.5 M solution in MeOH), and the mixture was stirred. After 5 min, 2-bromo-5-nitrothiazole (78 mg, 0.377 mmol) was added to the reaction mixture, which was stirred until the reaction was deemed complete by TLC (6 h). The reaction mixture was acidified with 1 N HCl, and the resulting precipitate was collected by filtration and washed with water (2 × 30 mL), hexanes (2 × 30 mL), and 10% ethyl acetate in hexanes (2 × 30 mL) to give a white solid. The residue was chromatographed over silica gel (40% ethyl acetate in hexane) to afford **3a** (102 mg, 71%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.27 (s, 9 H), 7.27 (d, $J$ = 8.4 Hz, 2 H), 7.47 (d, $J$ = 8.4 Hz, 2 H), 8.68 (s, 1 H); MS $m/z$ 777 (2M + Na)$^+$, 755 (2M + H)$^+$, 400 (M + Na)$^+$, 378 (M + H)$^+$, 354, 298, 250, 258, 207, 85, 74; HRMS calcd for $C_{15}H_{16}N_5O_3S_2$ (M + H) 378.0689, found 378.0690.

Following the above-mentioned procedure for **3a** and with the appropriate starting materials and reagents used, compounds **3b**−**q** were synthesized.

**4-(Benzo[*d*][1,3]dioxol-5-yl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3b).** Yield, 60%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 6.08 (s, 2 H), 6.84 (d, $J$ = 8.1 Hz, 1 H), 6.96 (s, 1 H), 7.01 (d, $J$ = 6 Hz, 1 H), 8.68 (s, 1 H); MS $m/z$ 387 (M + Na)$^+$, 365 (M + H)$^+$, 354, 349, 190, 158, 125, 102, 97; HRMS calcd for $C_{12}H_8N_5O_5S_2$ (M + H) 365.9961, found 365.9964.

**4-(4-Fluorophenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3c).** Yield, 62%; $^1$H NMR (300 DMSO-$d_6$) $\delta$ 7.28−7.35 (m, 2 H), 7.43−7.50 (m, 2 H), 8.65 (s, 1 H), 361 (M + Na)$^+$, 339 (M + H)$^+$, 294, 158, 85,74, 67, 60; HRMS calcd for $C_{11}H_7FN_5O_3S_2$ (M + H) 339.9969, found 339.9964.

**4-(2,4-Dimethoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3d).** Yield, 62%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.66 (s, 3 H), 3.79 (s, 3 H), 6.65 (d, $J$ = 8.4 Hz, 2 H), 7.18 (s, 1 H), 8.69 (s, 1 H); MS $m/z$ 785 (2M + Na)$^+$, 763 (2M + H)$^+$, 404 (M + Na)$^+$, 382 (M + H)$^+$, 359, 345, 158, 102, 85, 74, 55; HRMS calcd for $C_{13}H_{12}N_5O_5S_2$ (M + H) 382.0274, found 382.0281.

**4-(3,5-Dimethoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3e).** Yield, 67%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.70 (s, 6 H, 2 × OMe), 6.57 (s, 3 H), 8.69 (s, 1 H); HRMS calcd for $C_{13}H_{12}N_5O_5S_2$ (M + H) 382.0274, found 382.0280.

**4-(3,4-Dimethoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3f).** Yield, 64%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.67 (s, 3 H, OMe), 3.76 (s, 3 H, OMe), 6.88 (dd, $J$ = 8.4 and 1.8 Hz, 1 H), 6.98 (s, 1 H), 7.01 (d, $J$ = 8.4 Hz, 1 H), 8.68 (s, 1 H); HRMS calcd for $C_{13}H_{12}N_5O_5S_2$ (M + H) 382.0274, found 382.0282.

**4-(Naphthalen-1-yl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3g).** Yield, 61%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.38−7.76 (m, 5 H), 8.01−8.10 (m, 2 H), 8.53 (s, 1 H); MS $m/z$ 394 (M +

Na)$^+$, 372 (M + H)$^+$, 217, 169, 130, 107,85, 74, 67, 45; HRMS calcd for $C_{15}H_{10}N_5O_3S_2$ (M + H) 372.0220, found 372.0224.

**4-(2,5-Dimethoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3h).** Yield, 65%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.62 (s, 3 H, OMe), 3.67 (s, 3 H, OMe), 6.93 (s, 1 H), 7.06 (dd, $J$ = 8.4 and 1.8 Hz, 1 H), 7.11 (d, $J$ = 8.4 Hz, 1 H), 8.69 (s, 1 H); HRMS calcd for $C_{13}H_{12}N_5O_5S_2$ (M + H) 382.0274, found 382.0282.

**5-(5-Nitrothiazol-2-ylthio)-4-(4-(trifluoromethoxy)phenyl)-4*H*-1,2,4-triazol-3-ol (3i).** Yield, 67%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.39−7.57 (m, 4 H), 8.63 (s,1 H); MS $m/z$ 427 (M + Na)$^+$, 405 (M + H)$^+$, 364, 338, 306, 284, 192, 147, 106, 102, 97; HRMS calcd for $C_{12}H_7F_3N_5O_4S_2$ (M + H) 405.9886, found 405.9891.

**4-(4-Nitrophenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3j).** Yield, 60%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.74 (d, $J$ = 8.7 Hz, 2 H), 8.33 (d, $J$ = 8.7 Hz, 2 H), 8.62 (s, 1 H); MS $m/z$ 367 (M + H)$^+$, 360, 355, 85; HRMS calcd for $C_{11}H_6N_6O_5S_2$ 366.9914 (M + H), found 366.9900.

**4-(3,4-Difluorophenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3k).** Yield, 58%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.28−7.59 (m, 3 H), 8.64 (s, 1 H); MS $m/z$ 357 (M + H)$^+$, 356, 355, 288, 226, 174, 130, 125, 102, 84, 56; HRMS calcd for $C_{11}H_6F_2N_5O_3S_2$ (M + H) 357.9875, found 357.9866.

**4-(3-Fluorophenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3l).** Yield, 68%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.25−7.45 (m, 3 H), 7.49−7.56 (m, 1 H), 8.66 (s, 1 H); MS $m/z$ 361 (M + Na)$^+$, 339 (M + H)$^+$, 324, 229, 173, 158, 125, 116, 84, 74; HRMS calcd for $C_{11}H7FN_5O_3S_2$ (M + H) 339.9969, found 339.9970.

**4-(3-Methoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3m).** Yield, 61%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.72 (s, 3 H, OMe), 6.95 (d, $J$ = 8.1 Hz, 1 H), 7.01 (s, 1 H), 7.02 (d, $J$ = 8.1 Hz, 1 H), 7.37 (t, $J$ = 7.8 Hz, 1 H), 8.68 (s, 1 H); MS $m/z$ 725 (2M + Na)$^+$, 373 (M + Na)$^+$, 352 (M + H)$^+$, 338, 306, 284, 209, 201, 147, 106, 102, 97; HRMS calcd for $C_{12}H_{10}N_5O_4S_2$ (M + H) 352.0169, found 352.0177.

**4-(2-Methoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3n).** Yield, 69%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.69 (s, 3 H, OMe), 7.03 (t, $J$ = 7.5 Hz, 1 H), 7.18 (d, $J$ = 8.4 Hz, 1 H), 7.30 (d, $J$ = 7.8 Hz, 1 H), 7.47 (t, $J$ = 7.8 Hz, 1 H), 8.69 (s, 1 H); HRMS calcd for $C_{12}H_{10}N_5O_4S_2$ (M + H) 352.0169, found 352.0172.

**5-(5-Nitrothiazol-2-ylthio)-4-(3-(trifluoromethyl)phenyl)-4*H*-1,2,4-triazol-3-ol (3o).** Yield, 95%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.73−7.75 (m, 2 H), 7.83 (d, $J$ = 3.0 Hz, 1 H), 7.86 (s, 1 H), 8.66 (s, 1 H); MS $m/z$ 412 (M + Na)$^+$, 390 (M + H)$^+$, 159, 141, 129, 102, 98, 90, 74; HRMS calcd for $C_{12}H_6F_3N_5O_3S_2$ 389.9937 (M + H), found 389.9943.

**5-(5-Nitrothiazol-2-ylthio)-4-(4-(trifluoromethyl)phenyl)-4*H*-1,2,4-triazol-3-ol (3p).** Yield, 94%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.55 (d, $J$ = 9.0 Hz, 2 H), 8.02 (d, $J$ = 9 Hz, 2 H), 8.65 (s, 1 H); MS $m/z$ 412 (M + Na)$^+$, 390 (M + H)$^+$, 366, 221, 138, 74; HRMS calcd for $C_{12}H_6F_3N_5O_3S_3$ 389.9936 (M + H), found 389.9936.

**4-Cyclohexyl-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3q).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.01−1.26 (m, 3 H), 1.56−1.79 (m, 5 H), 2.01−2.10 (m, 2 H), 3.82−3.87 (m, 1 H), 8.75 (s, 1 H); MS $m/z$ 677 (2M + Na)$^+$, 350 (M + Na)$^+$, 328 (M + H)$^+$, 310, 306, 284, 281, 245, 204, 179, 138, 134, 129, 106, 100, 88, 60; HRMS calcd for $C_{11}H_{14}N_5O_3S_2$ (M + H) 328.0533, found 328.0548.

**4-(4-Methoxyphenyl)-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-ol (3r).** Yield, 71%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.76 (s, 3 H, OMe), 7.01 (d, $J$ = 8.7 Hz, 2 H), 7.30 (d, $J$ = 8.7 Hz, 2 H), 8.68 (s, 1 H); HRMS calcd for $C_{12}H_{10}N_5O_4S_2$ (M + H) 352.0169, found 352.0174.

Similarly, compounds **4a**−**d** were prepared using different aryl and alkyl bromides.

**5-(Butylthio)-4-phenyl-4*H*-1,2,4-triazol-3-ol (4a).** Yield, 68%; $^1$H NMR (300 MHz, $CDCl_3$) $\delta$ 0.90 (t, $J$ = 7.2 Hz, 3 H), 1.39 (sextet, $J$ = 7.8 Hz, 2 H), 1.66 (quintet, $J$ = 7.5 Hz, 2 H), 3.01 (t, $J$ = 7.5 Hz, 2 H), 7.35−7.57 (m, 5 H), 10.42 (br s, 1 H, OH); MS $m/z$ 272 (M + Na)$^+$, 250 (M + H)$^+$, 233, 190, 149, 102, 85; HRMS calcd for $C_{12}H_{16}N_3OS$ (M + H) 250.1009, found 250.1007.

**5-(Ethylthio)-4-phenyl-4*H*-1,2,4-triazol-3-ol (4b).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.35 (t, $J$ = 7.2 Hz, 3 H), 3.02 (t, q, $J$ = 7.2 Hz, 2 H), 7.35−7.56 (m, 5 H), 10.51 (br s, 1 H, OH); MS $m/z$ 244 (M + Na)$^+$, 222 (M + H)$^+$, 200, 149, 121, 85; HRMS calcd for $C_{10}H_{12}N_3OS$ (M + H) 222.0696, found 222.0697.

**5-(Benzylthio)-4-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)-4*H*-1,2,4-triazol-3-ol (4c).** Yield, 75%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.16 (s, 2 H), 4.26 (s, 4 H), 6.75 (d, $J$ = 8.4 Hz, 1 H), 6.82 (s, 1 H), 6.94 (d, $J$ = 8.7 Hz, 1 H), 7.22−7.31 (m, 5 H); MS $m/z$ 705 ((2M + Na)$^+$, 683 (2M + H)$^+$, 364 (M + Na)$^+$, 342 (M + H)$^+$, 326, 233, 158, 107, 87; HRMS calcd for $C_{17}H_{16}N_3O_3S$ (M + H) 342.0907, found 342.0912.

**5-(5-Nitrothiophen-2-ylthio)-4-phenyl-4*H*-1,2,4-triazol-3-ol (4d).** Yield, 71%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.06 (d, $J$ = 4.2 Hz, 1 H), 7.31−7.38 (m, 2 H), 7.41−7.49 (m, 3 H), 7.94 (d, $J$ = 4.5 Hz, 1 H); MS $m/z$ 321 (M + H)$^+$, 277, 233, 200, 149, 97, 85; HRMS calcd for $C_{12}H_9N_4O_3S_2$ (M + H) 321.0111, found 321.0116.

**2-Benzoyl-*N*-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)hydrazinecarbothioamide (5a).** To a suspension of sodium acetate (226 mg, 2.76 mmol) and benzoylhydrazine (532 mg, 2.76 mmol) in acetonitrile (5 mL) was added 2,3-dihydro-1,4-benzodioxin-6-yl isothiocyanate (532 mg, 2.76 mmol), and the reaction mixture was stirred for 24 h at room temperature. The solvent was removed by rotary evaporation, and the resulting crude was dissolved in 2 M NaOH solution. The filtrate was acidified with 1 N HCl. The precipitate was collected and washed with water (3 × 20 mL) and hexanes (2 × 20 mL) to afford **5a** as a white solid (873 mg, 96%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.23 (s, 4 H), 6.81 (m, 2 H), 6.99 (s, 1 H, NH), 7.49 (d, $J$ = 6.9 Hz, 1 H), 7.52 (d, $J$ = 7.8 Hz, 1 H), 7.58 (d, $J$ = 7.2 Hz, 1 H), 7.96 (d, $J$ = 6.9 Hz, 2 H), 9.62 (s, 1 H), 9.68 (s, 1 H, NH), 10.50 (s, 1H, NH); MS $m/z$ 352 (M + Na)$^+$, 330 (M + H)$^+$, 306, 296, 284, 179, 138, 100, 83; HRMS calcd for $C_{16}H_{15}N_3O_3S$ 330.0907 (M + H), found 330.0902.

**2-Benzoyl-*N*-(3-methoxyphenyl)hydrazinecarbothioamide (5c).** Yield, 78%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.73 (s, 3 H, OMe), 6.72 (d, $J$ = 7.8 Hz, 1 H), 7.04 (d, $J$ = 7.8 Hz, 1 H), 7.22 (t, $J$ = 8.4 Hz, 1 H), 7.45−7.59 (m, 3 H), 7.94 (d, $J$ = 7.5 Hz, 2 H), 9.72 (s, 1 H, NH), 9.73 (s, 1 H, NH), 10.53 (s, 1 H, NH).

**4-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (6a).** Compound **5a** (1.15 g, 3.50 mmol) in 2 M NaOH aqueous solution (35 mL) was stirred at reflux for 6 h. The reaction mixture was allowed to cool to room temperature and then acidified with 1 N HCl. The resulting precipitate was collected by filtration and washed with water (3 × 20 mL), hexanes (2 × 20 mL), and 1:1 hexanes/diethyl ether (20 mL) successively and dried in vacuo to afford **6a** as a white solid (655 mg, 60%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.29 (s, 4 H), 6.75 (dd, $J$ = 2.4, 8.1 Hz, 1 H), 7.93 (d, $J$ = 8.7 Hz, 1 H), 7.94 (s, 1 H), 7.34−7.45 (m, 5 H); MS $m/z$ 334 (M + Na)$^+$, 312 (M + H)$^+$, 280, 209, 179, 136, 100, 83; HRMS calcd for $C_{16}H_{13}N_3O_2S$ 312.0801 (M + H), found 312.0803.

**4-(3-Methoxyphenyl)-5-phenyl-4*H*-1,2,4-triazole-3-thiol (6c).** Yield, 71%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.72 (s, 3 H, OMe), 6.87 (d, $J$ = 7.8 Hz, 1 H), 7.02−7.08 (m, 2 H), 7.32−7.42 (m, 6 H); MS $m/z$ 306 (M + Na)$^+$, 284 (M + H)$^+$, 252, 149, 129, 106, 97, 85; HRMS calcd for $C_{15}H_{14}N_3OS$ (M + H) 284.0852, found 284.0854.

Compounds **6b**,**d**−**g** were commercially available.

**2-(4-(2,3-Dihydrobenzo[*b*][1,4]dioxin-6-yl)-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-5-nitrothiazole (7a).** To a solution of **6a** (173 mg, 0.556 mmol) in MeOH (1.4 mL) was added MeONa (1.3 mL, 0.5 M solution in MeOH) and stirred. After 5 min, 2-bromo-5-nitrothiazole (127 mg, 0.608 mmol) was added to the reaction mixture and stirred until the reaction was deemed complete by TLC (18 h). The reaction mixture was acidified with 1 N HCl, and the resulting precipitate was collected by filtration and washed with water (2 × 30 mL), haxanes (2 × 30 mL), and 10% ethyl acetate in hexanes (2 × 30 mL) to give a solid. The residue was chromatographed over silica gel (50% ethyl acetate in hexane) to afford the **7a** (167 mg, 68%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.29 (s, 4 H), 6.91 (dd, $J$ = 2.4, 7.8 Hz, 1 H), 6.89 (d, $J$ = 2.4 Hz, 1 H), 7.44−7.52 (m, 5 H), 8.73 (s, 1 H); MS $m/z$ 462 (M + Na)$^+$,

440 $(M + H)^+$, 160, 150, 142, 138, 125, 102, 98, 60; HRMS calcd for $C_{19}H_{13}N_5O_4S_2$ 440.0482 (M + H), found 440.0478.

Following the procedure as mentioned for **7a**, compounds **7b−g** were synthesized.

**2-(4-(2,4-Dichlorophenyl)-5-(1-methyl-1*H*-pyrrol-2-yl)-4*H*-1,2,4-triazol-3-ylthio)-5-nitrothiazole (7b).** Yield, 91%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.96 (s, 3 H), 5.59 (dd, $J$ = 1.5, 3.9 Hz, 1 H), 6.00 (dd, $J$ = 1.5, 3.6 Hz, 1 H) 7.06 (t, $J$ = 1.8 Hz, 1 H); MS $m/z$ 475 $(M + Na)^+$, 452 $(M + H)^+$, 397, 369, 338, 159, 110, 98, 79; HRMS calcd for $C_{1c}H_{10}Cl_2N_6O_2S_2$ 452.9756 (M + H), found 452.9759.

**2-(4-(3-Methoxyphenyl)-5-phenyl-4*H*-1,2,4-triazol-3-ylthio)-5-nitrothiazole (7c).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.71 (s, 3 H, OMe), 6.97 (d, $J$ = 7.8 Hz, 1 H), 7.10−7.16 (m, 2 H), 7.33−7.50 (m, 6 H), 8.70 (s, 1 H); MS $m/z$ 434 $(M + Na)^+$, 412 $(M + H)^+$, 364, 354, 298, 252, 141, 138, 129, 102, 60; HRMS calcd for $C_{18}H_{14}N_5O_3S_2$ (M + H) 412.0533, found 412.0541.

**2-(4-(Furan-2-ylmethyl)-5-(naphthalen-1-yl)-4*H*-1,2,4-triazol-3-ylthio)-5-nitrothiazole (7d).** Yield, 13%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 5.16 (s, 2 H), 6.00 (d, $J$ = 3.3 Hz, 1 H), 6.14 (d, $J$ = 2.4 Hz, 1 H), 7.38 (s, 1 H), 7.62−7.78 (m, 5 H), 8.10 (d, $J$ = 6.9 Hz, 1 H), 8.23 (d, $J$ = 6.9 Hz, 1 H), 8.73 (s, 1 H); MS $m/z$ 871 $(2M + H)^+$, 458 $(M + Na)^+$, 436 $(M + H)^+$, 326, 179, 102, 88, 84; HRMS calcd for $C_{20}H_{13}N_5O_3S_2$ 436.0533 (M + H), found 436.0543.

**5-Nitro-2-(4-phenyl-5-(thiophen-2-yl)-4*H*-1,2,4-triazol-3-ylthio)-thiazole (7e).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) 6.86−7.06 (m, 2 H), 7.53−7.74 (m, 6 H), 8.68 (s, 1 H); MS $m/z$ 409 $(M + Na)^+$, 387 $(M + H)^+$, 354, 338, 326, 310, 284, 179, 147, 138, 121, 106, 97, 60; HRMS calcd $C_{15}H_{10}N_5O_2S_3$ (M + H) 387.991, found 387.9996.

**5-Nitro-2-(4-phenyl-5-(pyridin-4-yl)-4*H*-1,2,4-triazol-3-ylthio)thiazole (7f).** Yield, 62%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.36 (d, $J$ = 6 Hz, 2 H), 7.50−7.61 (m, 5 H), 8.62 (d, $J$ = 5.4 Hz, 2 H), 8.69 (s, 1 H); MS $m/z$ 405 $(M + Na)^+$, 383 $(M + H)^+$, 359, 310, 226, 158, 141, 138, 129, 121, 109, 97, 90, 60; HRMS calcd for $C_{16}H_{11}N_6O_2S_2$ (M + H) 383.0379, found 383.0381.

**5-((4-Methyl-5-(5-nitrothiazol-2-ylthio)-4*H*-1,2,4-triazol-3-yl)methyl)-3-(pyrazin-2-yl)-1,2,4-oxadiazole (7g).** Yield, 79%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 1.24 (s, 2 H), 3.50 (s, 3 H), 8.78 (s, 1 H), 8.80 (s, 1 H), 8.87 (s, 2 H), 9.31 (s, 1 H); MS $m/z$ 871 $(2M + H)^+$, 458 $(M + Na)^+$, 436 $(M + H)^+$, 326, 179, 102, 88, 84; HRMS calcd for $C_{20}H_{13}N_5O_3S_2$ 436.0533 (M + H), found 436.0543.

**2,5-Bis(benzylthio)-1,3,4-thiadiazole (8a).** To a solution of 1,3,4-thiadiazole-2,5-dithiol (93 mg, 0.62 mmol) in MeOH (3 mL) was added MeONa (2.8 mL, 0.5 M solution in MeOH) and stirred. After 5 min, benzyl bromide (233 mg, 1.364 mmol) was added to the reaction mixture and stirred until the reaction was deemed complete by TLC (6 h). The reaction mixture was acidified with 1 N HCl, and the resulting precipitate was collected by filtration and washed with water (2 × 30 mL), haxanes (2 × 30 mL), and 10% ethyl acetate in hexanes (2 × 30 mL) to give a white solid. The residue was chromatographed over silica gel (30% ethyl acetate in hexane) to afford the **8a** (204 mg, 80%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.49 (s, 4 H), 7.25−7.45 (m, 10 H); MS $m/z$ 353 $(M + Na)^+$, 331 $(M + H)^+$, 192, 124, 102, 85; HRMS calcd for $C_{16}H_{15}N_2S_2$ (M + H) 331.0392, found 331.0398.

**2,5-Bis(4-fluorobenzylthio)-1,3,4-thiadiazole (8b).** Yield, 75%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.49 (s, 4 H), 7.11−7.19 (m, 4 H), 7.42−7.46 (m, 4 H); MS $m/z$ 389 $(M + Na)^+$, 367 $(M + H)^+$, 338, 284, 192, 147, 106, 102, 97, 85; HRMS calcd for $C_{16}H_{13}F_2N_2S_3$ (M + H) 367.0203, found 367.0208.

**2,5-Bis(4-methoxybenzylthio)-1,3,4-thiadiazole (8c).** Yield, 76%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 3.72 (s, 6 H), 4.43 (s, 4 H), 6.87 (d, $J$ = 8.4 Hz, 4 H), 7.32 (d, $J$ = 8.4 Hz, 4 H); MS $m/z$ 413 $(M + Na)^+$, 391 $(M + H)^+$, 305, 261, 226, 158, 149, 138, 106, 102, 97, 84; HRMS calcd for $C_{18}H_{19}N_2O_2S_3$ (M + H) 391.0603, found 391.0606.

**2,5-Bis(3-nitrobenzylthio)-1,3,4-thiadiazole (8d).** Yield, 73%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.64 (s, 4 H), 7.61(t, $J$ = 8.1 Hz, 2 H), 7.86 (d, $J$ = 7.8 Hz, 2 H), 8.12 (d, $J$ = 8.4 Hz, 2 H), 8.29 (s, 2H); HRMS calcd for $C_{16}H_{13}N_4O_4S_3$ (M + H) 421.0093, found 421.0095.

**2,5-Bis(4-nitrobenzylthio)-1,3,4-thiadiazole (8e).** Yield, 72%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.63 (s, 4 H), 7.68 (d, $J$ = 8.7 Hz, 4 H), 8.17 (d, $J$ = 8.7 Hz, 4 H); MS $m/z$ 442 $(M + Na)^+$, 421 $(M + H)^+$, 397, 364, 338, 306, 277, 201, 192, 147, 129, 118, 106, 102, 97; HRMS calcd for $C_{16}H_{13}N_4O_4S_3$ (M + H) 421.0093, found 421.0097.

**2,5-Bis(5-nitrothiazol-2-ylthio)-1,3,4-thiadiazole (8f).** Yield, 90%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.86 (s, 2H); MS $m/z$ 429 $(M + Na)^+$, 406 $(M + H)^+$, 227, 159, 102, 56; HRMS calcd for $C_8H_2N_6O_4S_5$ 406.8814 (M + H), found 406.8814.

**2,2′-(1,3,4-Thiadiazole-2,5-diyl)bis(sulfanediyl)dithiazole-5-carboxylic Acid (8g).** Yield, 78%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 8.36 (s, 2 H); MS $m/z$ 404 $(M + H)^+$, 306, 253, 233, 169, 146, 102, 95, 87, 85; HRMS calcd for $C_{10}H_5N_4O_4S_5$ (M + H) 404.8909, found 404.8908.

**5-(5-Nitrothiazol-2-ylthio)-1,3,4-thiadiazol-2-amine (9).** To a solution of 5-amino-1,3,4-thiadiazole-2-thiol (211 mg, 1.586 mmol) in MeOH (3 mL) was added MeONa (3.8 mL, 0.5 M solution in MeOH) and stirred. After 5 min, 2-bromo-5-nitrothiazole (331 mg, 1.586 mmol) was added to the reaction mixture and stirred until the reaction was deemed complete by TLC (12 h). The reaction mixture was acidified with 1 N HCl, and the resulting precipitate was collected by filtration and washed with water (2 × 30 mL), hexanes (2 × 30 mL), and 10% ethyl acetate in hexanes (2 × 30 mL) to give a white solid. The residue was chromatographed over silica gel (70% ethyl acetate in hexane) to afford the **9** (281 mg, 68%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.94 (br s, 2 H, $NH_2$), 8.74 (s, 1 H); MS $m/z$ 283 $(M + Na)^+$, 261 $(M + H)^+$, 229, 193, 158, 126, 97, 84, 47; HRMS calcd $C_5H_4N_5O_2S_3$ (M + H) 261.9522, found 261.9515.

**4-Fluoro-*N*-(5-(5-nitrothiazol-2-ylthio)-1,3,4-thiadiazol-2-yl)benzamide (10).** A solution of **9** (75 mg, 0.288 mmol), 4-fluorobenzoic acid (40 mg, 0.288 mmol), EDC (66 mg, 0.34 mmol), HOBt (47 mg, 0.34 mmol), DIEA (0.15 mL, 0.86 mmol) in DMF (2 mL) was stirred at 50 °C for 16 h. The reaction mixture was extracted with ethyl acetate (50 mL), washed with 10% $NaHCO_3$ solution (30 mL), brine (2 × 30 mL), and water (2 × 30 mL), dried ($MgSO_4$), and concentrated in vacuo. The residue was chromatographed over silica gel (30% ethyl acetate in hexane) to afford the **10** (82 mg, 75%). $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 7.42−7.46 (m, 2 H), 7.67 (s, 1 H, NH), 8.20−8.22 (m, 2 H), 8.87 (s, 1 H); MS $m/z$ 381 $(M-H)^+$, 306, 248, 204, 180, 174, 154, 138, 96; HRMS calcd for $C_{12}H_5FN_5O_3S_3$ (M − H) 381.9544, found 381.9535.

**5-(Benzylthio)-1,3,4-thiadiazol-2-amine (11).** Yield, 69%; $^1$H NMR (300 MHz, DMSO-$d_6$) $\delta$ 4.28 (s, 2 H), 7.11−7.19 (m, 2 H), 7.34−7.42 (m, 5 H, Ar−H, $NH_2$); MS $m/z$ 264 $(M + Na)^+$, 242 $(M + H)^+$, 192, 147, 102, 97, 85; HRMS calcd $C_9H_9H_9FN_3S_2$ (M + H) 242.0216, found 242.0215.

**DELFIA Assay (Dissociation Enhanced Lanthanide Fluoroimmuno Assay).** To each well of 96-well streptavidin-coated plates (Perkin-Elmer) 100 $\mu$L of a 100 ng/mL solution of biotin-labeled pep-JIP11 (biotin-lc-KRPKRPTTLNLF, where lc indicates a hydrocarbon chain of six methylene groups) was added. After 1 h of incubation and elimination of unbound biotin-pep-JIP11 by three washing steps, 87 $\mu$L of Eu-labeled anti-GST antibody solution (300 ng/mL, 1.9 nM), 2.5 $\mu$L of DMSO solution containing test compound, and 10 $\mu$L solution of GST-JNK for a final protein concentration of 10 nM were added. After 1 h of incubation at 0 °C, each well was washed five times to eliminate unbound protein and the Eu-antibody if displaced by a test compound. Subsequently, 200 $\mu$L of enhancement solution (Perkin-Elmer) was added to each well and fluorescence measured after 10 min of incubation (excitation wavelength, 340 nm; emission wavelength, 615 nm). Controls include unlabeled peptide and blanks receiving no compounds. Protein and peptide solutions were prepared in DELFIA buffer (Perkin-Elmer).

**In Vitro Kinase Assay.** The LanthaScreen assay platform from Invitrogen was utilized. The time-resolved fluorescence resonance

energy transfer assay (TR-FRET) was performed in 384-well plates. Each well received JNK (35 ng/mL), ATF2 (400 nM), and ATP (200 $\mu$M) in 50 mM HEPES, 10 mM $MgCl_2$, 1 mM EGTA, and 0.01% Brij-35, pH 7.5, and test compounds. The kinase reaction was performed at room temperature for 1 h, after which the terbium labeled antibody and EDTA were added into each well. After an additional hour of incubation, the signal was measured at 520/495 nm emission ratio on a fluorescence plate reader (Victor 2, Perkin-Elmer).

**Cell Based Assays for c-Jun.** The cell based kinase assays for c-Jun and ATF2 phosphorylation were carried out using the LanthaScreen c-Jun (1−79) Hela (Invitrogen, Carlsbad, CA) which stably expresses GFP-c-Jun 1−79. Phosphorylation was determined by measuring the time-resolved FRET (TR-FRET) between a terbium labeled phosphospecific antibody and the GFP-fusion protein.[12] The cells were plated in white tissue culture treated 384-well plates at a density of 10 000 cells per well in 32 $\mu$L of assay medium (Opti-MEM, supplemented with 1% charcoal/dextran-treated FBS, 100 U/mL penicillin, 100 $\mu$g/mL streptomycin, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 25 mM HEPES, pH 7.3, and lacking phenol red). After overnight incubation, cells were pretreated for 60 min with compound (indicated concentration) followed by 30 min of stimulation with 2 ng/mL of TNF-$\alpha$, which stimulates both JNK and p38. The medium was then removed by aspiration, and the cells were lysed by adding 20 $\mu$L of lysis buffer (20 mM Tris-HCl, pH 7.6, 5 mM EDTA, 1% NP-40 substitute, 5 mM NaF, 150 mM NaCl, 1:100 protease and phosphatase inhibitor mix, SIGMA P8340 and P2850, successively). The lysis buffer included 2 nM terbium labeled antipc-Jun (pSer73) detection antibodies (Invitrogen). After allowing the assay to equilibrate for 1 h at room temperature, TR-FRET emission ratios were determined on a BMG Pherastar fluorescence plate reader (excitation at 340 nm, emission at 520 and 490 nm; 100 $\mu$s lag time, 200 $\mu$s integration time, emission ratio = Em520/Em 490).

**Molecular Modeling.** Molecular modeling studies were conducted on a Linux workstation and a 64 3.2-GHz CPUs Linux cluster. Docking studies were performed using the X-ray coordinates of JNK1 (PDB code 2H96).[25] The complexed JIP peptide was extracted from the protein structure and was used to define the binding site for docking of small molecules. The genetic algorithm (GA) procedure in the GOLD docking software performed flexible docking of small molecules, whereas the protein structure was static.[26−30] For each compound, 20 solutions were generated and subsequently ranked according to Chemscore.[26] The protein surface was prepared with the program MOLCAD as implemented in Sybyl and was used to analyze the binding poses for studied small molecules.[26−30]

**In Vivo Experiments. Insulin Tolerance Test.** Eleven-week-old male BKS.Cg−+Lepr$^{db}$/+Lepr$^{db}$/OlaHsd db/db mice from Harlan (Harlan Sprague Dawley, Inc.; Indianapolis, IN) were randomized on the basis of blood glucose levels acclimated 3 days prior to drug dosing. Blood glucose was read using a hand-held glucose meter (OneTouch Ultra, LifeScan, a Johnson & Johnson Company, U.K.) after tail snipping. Mice were fasted 6 h before intraperitoneal (ip) administration of 25 mg/kg compounds **9**, **7b**, and **8f**. Thirty minutes after test article administration, bovine insulin (Sigma, I-0516 at 0.75 mg/kg) was administered via ip injection. Blood samples were taken at designated time points, and blood glucose levels were measured as previously described. Food was returned 3 h after test article administration.

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**Supporting Information Available:** Proton NMR spectra, HPLC traces of key compounds (purity), elemental analysis data of key compounds, and supporting figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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