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Design, Synthesis, and Structure-Activity Relationship Studies of Thiophene- 3-carboxamide Derivatives as Dual Inhibitors of the c-Jun N-Terminal Kinase

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Abstract

We report comprehensive structure activity relationship studies on a novel series of c-Jun N-terminal kinase (JNK) inhibitors. Intriguingly, the compounds have a dual inhibitory activity by functioning as both ATP and JIP mimetics, possibly by binding to both the ATP binding site and to the docking site of the kinase. Several of such novel compounds display potent JNK inhibitory profiles both *in vitro* and in cell.

The c-Jun N-terminal kinases (JNKs) have been originally identified in the early 1990s as a family of serine/threonine protein kinases that are activated by a range of stimuli. The activation of each of the JNK genes results in the phosphorylation of the N-terminal transactivation domain of the c-Jun transcription factor.[1–4] Three JNK isoforms (JNK1, 2 and 3) share more than 90% amino acid sequence identity and the ATP pocket is highly conserved (>98% identities). These proteins are often activated in response to a large variety of cellular stresses including irradiation, hypoxia, peroxides, heat shock, and chemotoxins as well as various cytokines, thus participating in the onset of apoptosis.[5,6] It has been clearly established that excessive up-regulation of JNK activity results or is associated with a number of human disorders including type-2 diabetes and obesity, neurodegeneration and stroke, cancer and inflammation.[1–3] Hence, JNK inhibitors are expected to be viable agents to devise novel therapies against these diseases, and there have been large efforts in identifying small molecule JNK inhibitors targeting its ATP binding site.[7–13]

Peculiar to JNKs substrates and scaffold proteins, is a JNK interacting conserved consensus sequence R/KXXXXLXL termed the D-domain.[14,15] A short peptide corresponding to the D-domain of the scaffolding protein JIP-1 (aa 153–163; pep-JIP1) has been shown to inhibit JNK activity *in vitro*, displaying noteworthy selectivity with little inhibition of the closely related Erk and p38 MAPKs.[16–19] Furthermore, recent *in vivo* data, generated for studies

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Supporting Information Available

Detailed synthetic procedures and analytical data are available for the reported compounds. A dose response curve for the inhibition of JNK in cell is also reported. Docking studies with compounds **19** and **39** are also included.

focusing on pep-JIP1 fused to the cell permeable HIV-TAT peptide, show that its administration in various mice models of insulin resistance and type-2 diabetes restores normoglycemia without causing hypoglycemia.[20] Despite these encouraging data, peptide's instability *in vivo* may hamper the development of novel JNK-related therapies based on such peptides.[16–20]

Based on these premises, 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 its JIP binding site rather than the highly conserved ATP binding site of the protein. Very recently, we have reported the identification of 5-(5-nitrothiazol-2-ylthio)-1,3,4-thiadiazol-2-amine series[21] related to compound **BI-78D3**[22] (Figure 1), as initial JIP mimetic inhibitors. These compounds were discovered using a displacement assay with a biotinylated-pepJIP1 peptide and employing a DELFIA assay platform in a medium size screening campaign.[22] In our continued interest in the development of JNK inhibitors[21–23] we now report further structure-activity relationship studies describing novel small molecules thiophene-carboxamide derivatives as JNK inhibitors targeting its JIP/substrate docking site. Intriguingly, we believe that the compounds are also able to function as ATP mimetics for JNK, which makes them particularly interesting. The 4,5-dimethyl-2-(2-(naphthalen-1-yl)acetamido)thiophene-3-carboxamide (**1**, Figure 1) was qualified as a hit and became the starting point of our medicinal chemistry efforts, with an IC_{50} value for the displacement of pepJIP1 in the DELFIA assay of 15.8 μM, inhibiting JNK1 kinase activity in the Lantha assay platform with an IC_{50} value of 26.0 μM. To investigate the effects on potency induced by small changes in the structure of **1**, we developed the general synthetic route for the preparation of this series. A variety of commercially available 2-aryl acetic acids were treated with aryl 2-amino-3-carboxamides in the presence of EDC at room temperature to give **5a–5g** and **11–74** (Schemes 1, 2, and 3) in moderate to good yields. Replacement of the thiophene moiety with a phenyl ring led to compound **3** that showed a drastic drop in activity (IC_{50} > 100 μM), similarly replacing the 3-carboxamide group on the thiophene with an acid, resulting in compound **5a**, or an ester, resulting in compound **5b**, or a cyano group, as in compound **5c**, also resulted in a significant loss of JNK1 inhibitory activity (Table 1). The position of carboxamide is also important for JNK1 inhibitory activity as the analogue with the carboxamide at the 5-position on the thiophene (compound **5f**) was completely inactive. The 4-methyl (**5d**) or 5-methyl (**5e**) or 4,5-dimethyl substitutions on the thiophene of compound **1** also resulted in less active compounds (IC_{50} > 25 μM), compared to the un-substituted compound (**5g**, IC_{50} = 5.4 μM). Therefore, we retained 4 and 5-positions unsubstituted and carboxamide on the 3-position on the thiophene, and explored modifications at the 2-position. We observed that introducing substituents with one carbon linker did not affect the inhibitory properties of the series (i.e. compound **7**, IC_{50} = 3.6 μM versus compound **8**, no linker, IC_{50} = 5.9 μM), while longer chains (i.e. compound **9** with a 2-carbon linker, IC_{50} > 100 μM, or compound **10** with a trans-2-carbon linker, IC_{50} > 100 μM) are not tolerated (Table 1). Based on these observations, we synthesized additional analogs of compound **7** with a variety of aryl or heteroaryl substitutions (Scheme 3). The mono fluoro or difluoro substitutions (compounds **29**, **30**, **31**, **52**, **53**, **54**, **55**, **56**, and **71**) on the benzene ring were well tolerated (IC_{50} = 8.3 μM, 9.4 μM, 5.1 μM, 8.2 μM, 10.2 μM, 9.7 μM, 7.4 μM, 5.8 μM, 4.9 μM respectively) but the penta-fluoro benzene, compound **60**, was inactive (IC_{50} > 50 μM). Similarly, a chlorine on positions 2 and 3 on the benzene ring resulted in compounds with good inhibitory activities (compound **26**, IC_{50} = 1.4 μM and compound **27,** IC_{50} = 2.6 μM). However a chlorine (**28**, IC_{50} = 18.7 μM) or larger substituents such as bromine (**34**, IC_{50} > 25 μM), isopropyl (**50**, IC_{50} > 25 μM), phenyl (**62**, IC_{50} > 50 μM), and isobutyl (**20**, IC_{50} > 25 μM) in the 4-position on the benzene ring were not tolerated, whereas similar substitutions in positions 2 or 3 resulted in compounds with good inhibitory properties against JNK1. Furthermore, introducing potential hydrogen bonding acceptors such as methoxy (compound

37, IC_{50} = 11.9 μM, compound **38,** IC_{50} = 2.6 μM and compound **39**, IC_{50} = 5.7 μM), methylene dioxy (compound **19**, IC_{50} = 1.8 μM), and benzodioxane (compound **18** IC_{50} = 2.7 μM) groups on the benzene ring resulted in compounds with significant JNK1 inhibition while other polar groups, such as a morpholine or a piperazine (compounds **64**, **65**, **67,** and **68**) resulted in compounds with poor inhibitory activities (IC_{50} > 50 μM), except for compound **66** in which some activity was retained (IC_{50} = 7.6 μM). Finally, we found that replacing the benzene with a benzothiophene (compound **25**, Figure 1) resulted in a significant improvement with respect to both JNK1 kinase activity inhibition and JNK1/pepJIP displacement.

Subsequently, we performed dose response measurements and enzyme kinetics assays with compound **25**. Compound **25** showed an IC_{50} of 1.32 μM in the kinase assay (Figure 2) and an IC_{50} of 4.62 μM in displacing pepJIP1, as measured by DELFIA assay, that indicates that the compound may bind directly at JIP site (Figure 2). However, enzyme kinetics experiments suggested that compound **25** is an ATP competitive inhibitor as well as a mixed substrate competitive inhibitor (Figure 2). This is quite intriguing, suggesting that the compound may bind independently to both the ATP and the JIP binding sites on JNK. Interestingly, our findings are similar to what recently reported by Chen *et al.*[24] that have identified a similar dual JIP site and ATP site JNK inhibitor based on kinetic experiments.

Hence, to determine the selectivity, compound **25** was tested against a panel of 26 kinases. Compound **25** was found to be nearly inactive (Table 2) against p38α (2% inhibition at 25 μM), a member of the MAPK family with high structural similarity to JNK (52% sequence identity, considering the kinase domains), and inactive against other MAP kinases and lipid kinases (Table 2), further corroborating that these compounds may elicit their activity by binding not only to the highly conserved ATP site but also the JNK docking site. This compound showed a preferential inhibition of JNK1 and JNK2 over JNK3 (Table 2), in agreement with our previous findings with compound **BI-78D3**[22] (Figure 1) and with the reported data on pepJIP1.[16–20]

Finally, to assess the activity of the compounds in cell we employed a cell-based LanthaScreen™ kinase assay. In this assay, compound **25** is able to inhibit TNF-α stimulated phosphorylation of c-Jun with an IC_{50} value of 7.5 μM (Supporting Information), which parallels well the *in vitro* activity and previous findings with this type of assay and other JNK inhibitors.[22]

To shed light into the possible binding mode of these compounds, we performed molecular modeling studies,[25–27] using the ATP and JIP binding pockets derived from the X-ray crystal structure of the ternary complex including JNK1, pepJIP1, and the ATP mimic SP600125 (PDB ID 1UKI). From the docked binding pose, compounds of this series, represented by compound **25** (Figure 3) appeared to be deeply inserted in the ATP binding site and further stabilized by hydrogen-bonding interactions involving its carboxamide NH_2 and side-chain atoms of the residue Gln 37. The docked poses of compounds **18**, **19** and **38** suggest the presence of additional hydrogen bonding interactions with the backbone amide of Met 111 in the ATP binding site, providing a justification for the increased inhibitory properties for these compounds (Supporting Information).

Furthermore, the JIP1 docking site seemed also able to potentially accommodate compounds of this series, as shown for compound **25** (Figure 3) through hydrophobic contacts and hydrogen bonding interactions between the carboxamide –CO– and $–NH_2$ groups with the backbone NH of Val 118 and the backbone carbonyl of Asn 114, respectively. Hence, both binding modes would reflect the observed SAR including the direct ability of the

compounds to displace the pepJIP1/JNK interactions in the DELFIA assay, suggesting that the dual inhibition is possible also on a theoretical basis.

To further corroborate this hypothesis, we conducted isothermal titration calorimetry experiments to monitor the thermodynamics of bindings of compound **25** to JNK2 in presence and absence of saturating concentrations of either a non-hydrolizable ATP or of pepJIP1. These studies revealed indeed that compound **25** binds to JNK2 with a K_d value of 640 nM with a 2:1 ligand to protein stoichiometry, and that the binding decreases significantly in presence of either the non-hydrolizable ATP or pepJIP1. The measured K_d values for compound **25** to JNK1 under these saturating conditions were 1.1 μM (in presence of ATP) and 3.1 μM (in presence of pepJIP1), both with a 1:1 ligand to protein stoichiometry.

Finally, to assess basic pharmacological properties of the proposed thiophene carboxamide derivatives, we determined *in vitro* plasma and microsomal stability for selected compounds. Compounds **25**, **33**, and **26** remained basically unaltered after 60 min of incubation in rat plasma while were degraded 85% ($T_{1/2}$ 18 min), 74% ($T_{1/2}$ 24 min), and 70% ($T_{1/2}$ 27 min) respectively, after 60 min of incubation in rat microsomal preparations, data suggesting that the molecules might be suitable for further optimizations and *in vivo* studies. Thiophenes are considered potential toxicophores due to their metabolites that can causehepatotoxicity, immunotoxicity and nephrotoxicity. [28, 29,30] However, di-substitutions or ring deactivation by addition of adjacent functionalities, have been proven to minimize metabolic oxidation hence reducing toxicity. [28,30] Hence, the observed relatively long microsomal half-lives of these compounds can be attributed to the presence of di-substitutions on the thiophene ring. Finally, two very recent reports, describing the pharmacology of other thiophene-based JNK inhibitors, albeit with rather different structures form those reported herein, corroborated the present observations [31,32].

In summary, we report data on a series of novel JNK inhibitors that resulted in the selection of compound **25**, that apparently inhibits JNK by possibly binding to both the ATP and JIP binding site, independently. Because the compound possesses a good balance of potency, selectivity, solubility, metabolic stability, and cellular activity, we anticipate that this series may hold promise for further development of novel JNK inhibitors as chemical probes and eventually for therapeutic purposes.

Methods

Synthetic chemistry

Unless otherwise indicated, all anhydrous solvents were commercially obtained and stored in Sure-seal 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 Varian 300 or 500 MHz instruments. Chemical shifts (δ) are reported in parts per million (ppm) referenced to Me_4Si. 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 key compounds was obtained in a HPLC Breeze from Waters Co. using an Atlantis T3 3μm 4.6×150 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 λ=254 nm. Purity of key compounds was established by elemental analysis as performed on a Perkin Elmer series II-2400. Combustion analysis was performed by NuMega Resonance labs, Inc., San Diego,

CA. Details on the experimental procedures and key analytical data for each of the reported compound are available as supplementary material.

Plasma Stability Assay

Test compound solution was incubated (1 μM, 2.5% final DMSO concentration) with fresh rat plasma at 37 °C. The reactions were terminated at 0, 30, and 60 min by the addition of two volumes of methanol containing internal standard. Following protein precipitation and centrifugation, the samples were analyzed by LC-MS. The percentage of parent compound remaining at each time point relative to the 0 min sample is calculated from peak area ratios in relation to the internal standard. Compounds were run in duplicate with a positive control known to be degraded in plasma.

Microsomal Stability Assay (RLM assay)

Solutions of three compounds were incubated with RAT liver microsomes (RLM) for 60 minutes at 37.5 °C. The final incubation solutions contained 4 μM test compound, 2 mM NADPH, 1 mg/ml (total protein) microsomes, and 50 mM phosphate (pH 7.2). Compound solutions, protein, and phosphate were pre-incubated at 37.5 °C for 5 min and the reactions were initiated by the addition of NADPH and incubated for 1 hour at 37.5 °C. Aliquots were taken at 15 minute time-points and quenched with the addition of methanol containing internal standard. Following protein precipitation and centrifugation, the samples were analyzed by LC-MS. Test compounds were run in duplicate with 2 control compounds of known half life.

DELFIA Assay (dissociation enhanced lanthanide fluoro-immuno assay)

To each well of 96-well streptavidin-coated plates (Perkin-Elmer) 100 μL of a 100 ng/ml solution of biotin-labeled pep-JIP11 (Biotin-lc-KRPKRPTTLNLF, where lc indicates a hydrocarbon chain of 6 methylene groups) was added. After 1 hr incubation and elimination of unbound biotin-pep-JIP11 by 3 washing steps, 87 μL of Eu-labeled anti-GST antibody solution (300ng/ml; 1.9 nM), 2.5 μL DMSO solution containing test compound, and 10 μL solution of GST-JNK1 for a final protein concentration of 10 nM was added. After 1 hr incubation at 0 °C, each well was washed 5 times to eliminate unbound protein and the Eu-antibody if displaced by a test compound. Subsequently, 200 μL of enhancement solution (Perkin-Elmer) was added to each well and fluorescence measured after 10 min 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 JNK1 (35 ng/mL), ATF2 (400 nM), and ATP (0.2 μM) in 50 mM HEPES, 10mM MgCl 2, 1mM EGTA and 0.01% Brij-35, pH 7.5 and test compounds. The kinase reaction was performed at room temperature for 1 hr. After which, the terbium labeled antibody and EDTA were added into each well. After an additional hour incubation, the signal was measured at 520/495 nm emission ratio on a fluorescence plate reader (Victor 2, Perkin-Elmer).

Isothermal Titration Calorimetry

Titrations were done using a VP-ITC calorimeter from Microcal (Northampton, MA). Depending upon the solubility of the titrants, JNK2 was used at 25–50 μM in 20 mM sodium phosphate buffer (pH 7.4), 5% DMSO, and 0.01% triton X-100. Titrants were used at 15× protein concentration (375–750 μM) in the same buffer. For competition titrations, 350 μM **25** was titrated to 25μM JNK2 in the presence of 250 μM ATPγS (Biomol International, Plymouth Meeting, PA) or pepJIP1 (ie. 250 μM ATPγS or pepJIP1 was present in both the syringe and cell of the ITC). Titrations of compound alone into buffer were done to determine the heats of dilution of compounds and were negligible compared to compound to protein titrations. Compound heats of dilution were subtracted from compound/protein titrations. Titrations were carried out at 25 °C. Data were analyzed using Microcal Origin software provided by the ITC manufacturer.

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 1UKI). 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[25] docking software performed flexible docking of small molecules whereas the protein structure was static and compound poses minimized according to the GOLD scoring function.[25] For each compound, 20 geometries were generated and the solution with the best fit according to the scoring function[25] was used to represent the docked pose. 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.

Cell based assays for c-Jun phosphorylation

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 express GFP-c-Jun 1–79. Phosphorylation was determined by measuring the time resolved FRET (TR-FRET) between a terbium labeled phospho-specific antibody and the GFP-fusion protein. The cells were plated in white tissue culture treated 384 well plates at a density of 10000 cell per well in 32 μl assay medium (Opti-MEM®, supplemented with 1% charcoal/dextran-treated FBS, 100 U/mL penicillin and 100 μg/mL streptomycin, 0.1 mM non-essential 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 μl 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 respectively). The lysis buffer included 2 nM of the terbium labeled anti-pc-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 520 nm and 490 nm; 100 μs lag time, 200 μs integration time, emission ratio = Em520 / Em 490).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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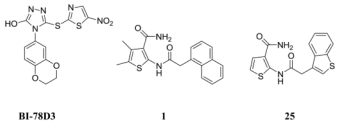


Figure 1.
Previously reported key JIP1 site binding JNK inhibitor (**BI-78D3**) and thiophene 3-carboxamide inhibitors indentified via HTS (**1** and **25**).

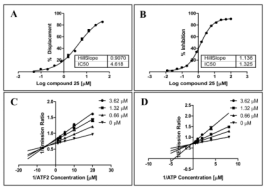


Figure 2.

Dose response curves for the displacement of pepJIP1 from JNK1 by compound **25** in the DELFIA assay (**A**), and for the inhibition of JNK1 kinase activity in the LANTHA assay by compound **25** (**B**). Lineweaver-Burk analyses for the inhibition of JNK1 kinase activity (LANTHA assay) by compound **25** with increasing substrate ATF2 (**C**) or ATP (**D**) concentrations. Data were collected in triplicates.

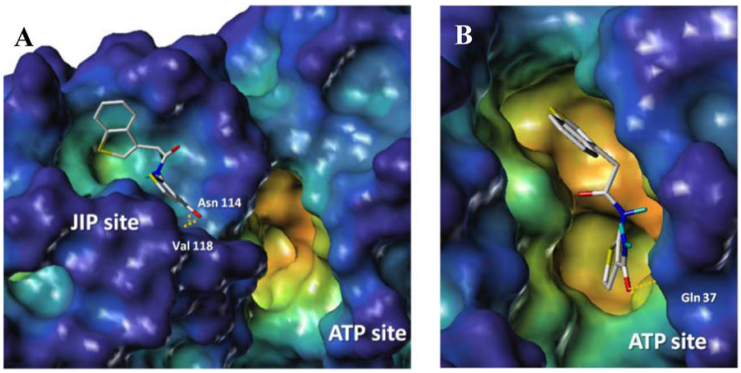
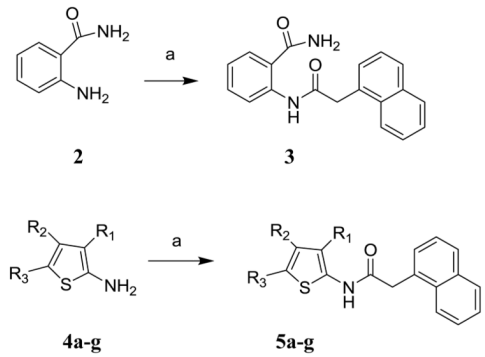


Figure 3.

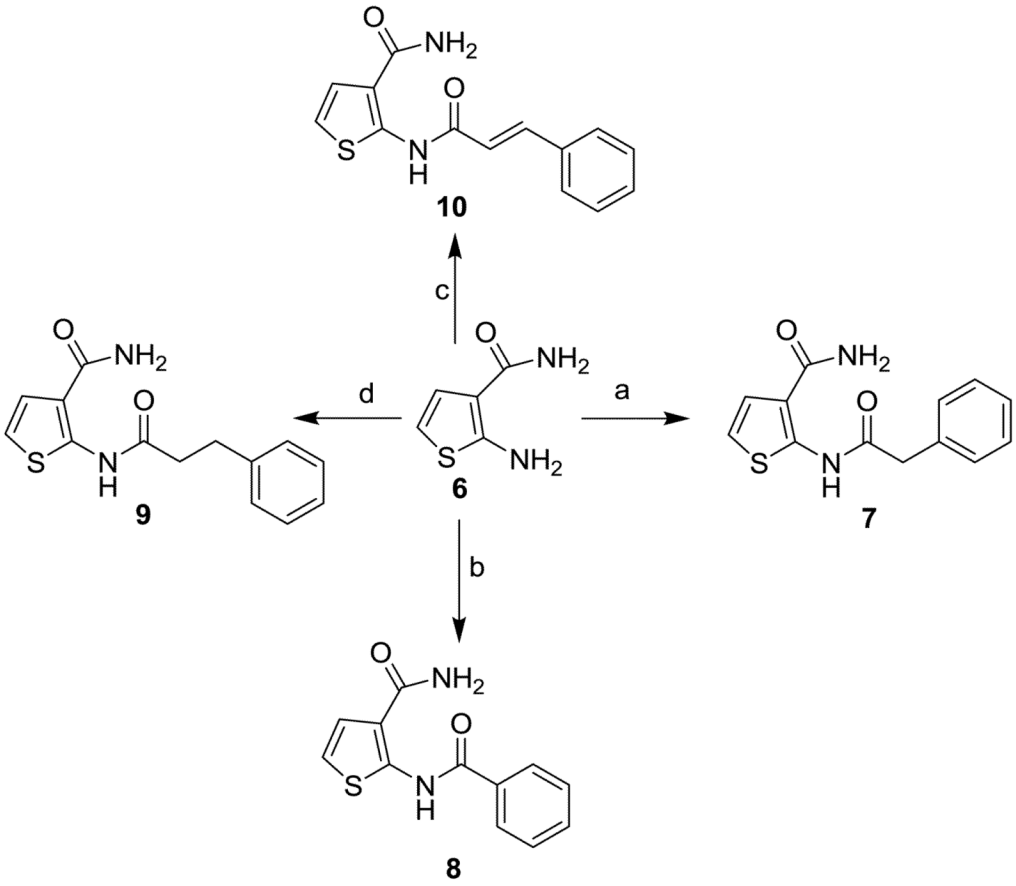
Docking studies of compound **25** within the JIP (**A**) and ATP (**B**) binding sites of JNK1 (PDB ID 1UKI). The surface was generated with MOLCAD[27] and color coded according to cavity depth (blue, shallow; yellow, deep). Predicted hydrogen bonding interactions are highlighted with yellow dashed lines.



a: R_1 = carboxylic acid, $R_2 = R_3 = H$; **b**: R_1= methyl ester, $R_2 = R_3$= H; **c**: R_1= cyano, $R_2 = R_3$= H; **d**: R_1 = carboxamide, R_2 = Me, R_3 = H; **e**: R_1 = carboxamide, R_2 = H, R_3 = Me; **f**: R_1 = H, R_2 = H, R_3 = carboxamide; **g**: R_1= carboxamide, $R_2 = R_3$= H.

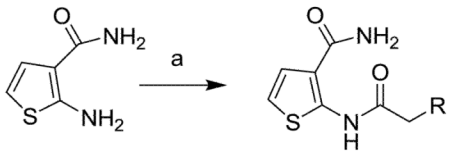
Scheme 1.

Reagents and conditions: (a) 2-(1-Naphthyl) acetic acid, EDC, HOBt, DIEA, DMF, room temp.



Scheme 2.

Reagents and conditions: (a) phenyl acetic acid, EDC, HOBt, DIEA, DMF, room temp; (b) benzoic acid, EDC, HOBt, DIEA, DMF, room temp.; (c) 3-phenylpropanoic acid, EDC, HOBt, DIEA, DMF, room temp.; (d) *trans* cinnamic acid, EDC, HOBt, DIEA, DMF, room temp.



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11: R = 2-bromo-6-methoxy-1-naphthyl; **12:** R = 2-methoxy-6-bromo-1-naphthyl; **13:** R = 2-naphthyl; **14:** R = thiophene-2-yl; **15:** R = 3,5-dimethylphenyl; **16:** R = pyrene-1-yl; **17:** R = 4-thiopheny-2-yl-thiazol-2-yl; **18:** R = 3,4-benzodioxane; **19:** R = 3.4-methylenedioxyphenyl; **20:** R = 4-isobutylphenyl; **21:** R = 2-pheny-thiazol-4-yl; **22:** R = 2-methoxy-5-methylphenyl; **23:** R = benzofuran-3-yl; **24:** R = benzoimidazol-2-yl; **25:** R = benzothiophen-3-yl; **26:** R = 2-chlorophenyl; **27:** R = 3-chlorophenyl, **28:** R = 4-chlorophenyl, **29:** R = 2-fluorophenyl, **30:** R = 3-fluorophenyl, **31:** R = 4-flurophenyl; **32:** R = 2-bromophenyl; **33:** R = 3-bromophenyl; **34:** R = 4-bromophenyl; **35:** R = 2-iodophenyl; **36:** R = 3-iodophenyl; **37:** R = 2-methoxyphenyl; **38:** R = 3-methoxyphenyl; **39:** R = 4-methoxyphenyl, **40:** R = 2-methylphenyl; **41:** R = 3-methylphenyl; **42:** R = 4-methylphenyl; **43:** R = 3-nitrophenyl; **44:** R = 4-nitrophenyl; **45:** R = 3-trifluoromethylphenyl; **46:** R = 4-trifluorophenyl; **47:** R = 4-trifluoromethoxyphenyl; **48:** R = 3-cyanophenyl; **49:** R = 4-cyanophenyl; **50:** R = 4-isopropyl; **51:** R = 4-ethoxyphenyl; **52:** R = 3,5-difluorophenyl; **53:** R = 3,4-difluorophenyl; **54:** R = 2,4-difluorophenyl; **55:** R = 2,6-difluorophenyl; **56:** R = 2,5-difluorophenyl; **57:** R = 2-chloro-6-fluorophenyl; **58:** R = 3,4-dimethoxyphenyl; **59:** R = 3,5-dimethoxyphenyl; **60:** R = 2,3,4,5,6-pentafluorophenyl; **61:** R = 3-pyridyl; **62:** R = 4,4-biphenyl; **63:** R = indol-3-yl; **64:** R = morpholino; **65:** R = 2,6-dimethylmorpholino; **66:** R = 4-furan-2-yl-piperazin-1-yl; **67:** R = 4-cyclopropane-carbonyl-piperazin-1-yl; **68:** R = (4-(2-(trifluoromethyl)phenylsulfonyl)piperazin-1-yl); **69:** R = 5,6,7,8-tetrahydronaphthalen-2yl; **70:** R = 2,3-dihydro-1H-inden-5-yl; **71:** R = 6-fluoro-4H-benzo[d][1,3]dioxin-8-yl; **72:** R = (2-(6-(thiophen-2-yl)imidazo[2,1-b]thiazol-3-yl); **73:** R = (2-(9-chloro-3,4-dihydro-2H-benzo[b][1,4]dioxepin-7-yl); **74:** R = (2-(5-methylimidazo[2,1-b]thiazol-2-yl).

Scheme 3.
Reagents and conditions: (a) 2-aryl-acetic acids, EDC, HOBT, DIEA, DMF, room temp.

Table 1

Inhibition results for thiophene carboxamide series against JNK-1.

Compd.	Kinase activity assay $IC_{50}{}^{a}$(μM)	Compd.	Kinase activity assay $IC_{50}{}^{a}$(μM)	Compd.	Kinase activity assay $IC_{50}{}^{a}$(μM)
1	26.0	**24**	>25	**50**	>25
3	>100	**25**	1.3	**51**	1.9
5a	>100	**26**	1.4	**52**	8.2
5b	>100	**27**	2.6	**53**	10.2
5c	>100	**28**	18.7	**54**	9.7
5d	>25	**29**	8.3	**55**	7.4
5e	>25	**30**	9.4	**56**	5.8
5f	>100	**31**	5.1	**57**	12.5
5g	5.4	**32**	2.9	**58**	>25
7	3.6	**33**	1.8	**59**	3.1
8	5.9	**34**	>25	**60**	>50
9	>100	**35**	3.4	**61**	8.1
10	>100	**36**	1.8	**62**	>50
11	>25	**37**	11.9	**63**	9.4
12	>25	**38**	2.6	**64**	>50
13	>25	**39**	5.7	**65**	65.3
14	3.2	**40**	6.0	**66**	7.6
15	2.7	**41**	5.4	**67**	83.5
16	>50	**42**	4.3	**68**	75.1
17	>25	**43**	>25	**69**	3.6
18	2.7	**44**	2.9	**70**	2.5
19	1.8	**45**	7.6	**71**	4.9
20	>25	**46**	6.4	**72**	2.5
21	>25	**47**	5.8	**73**	1.9
22	2.30	**48**	20.9	**74**	2.5
23	>25	**49**	3.4		

[a] Values are means of at least three or more experiments. Standard deviation generally within ±25%

Table 2

Selectivity profile for compound **25** [a,b]

Kinase	% inhibition at 25 μM	Kinase	% inhibition at 25 μM	Kinase	% inhibition at 25 μM
JNK1	79%[c]	BRAF	2%	MAPK14 (p-38α)	11%
JNK2	72%[c]	CAMK1	2%	NEK1	0%
JNK3	18%[c]	CDK1	3%	PDK1 direct	0%
MAPK14 (p-38α, direct)	2%	CHEK1 (CHK1)	0%	PI3Kα	4%
MAPK3 (ERK1)	3%	CHUK (IIKα)	0%	PIM1	0%
MAPK1 (ERK2)	4%	FRAP1 (mTOR)	0%	PRKCA (PKCα)	0%
AKT1	5%	GSK3α	16%	ROCK1	0%
AMPK A1	0%	MAK2K1 (MEK1)	0%	SPHK1	1%
AURK A1	0%	MAPK3K9 (MLK1)	0%	MAP4K2 (GCK)	1%

[a] From Invitrogen (Life Technologies) kinase profiling service;

[b] Values are means of at least two independent experiments;

[c] Z'-LYTE assay.