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Synthesis and SAR of piperazine amides as novel c-jun N-terminal kinase (JNK) inhibitors

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ABSTRACT

A novel series of c-jun N-terminal kinase (JNK) inhibitors were designed and developed from a high-throughput-screening hit. Through the optimization of the piperazine amide **1**, several potent compounds were discovered. The X-ray crystal structure of **4g** showed a unique binding mode different from other well known JNK3 inhibitors.

The c-jun N-terminal kinase (JNKs) are serine/threonine protein kinases and members of the mitogen-activated protein kinase (MAPK) family which can be activated in response to various stimuli such as environmental stress, cytokines and fatty acids.[1] Activated JNK phosphorylates both cytoplasmic substrates (cytoskeletal proteins, Bcl-2, APP) as well as nuclear transcription factors (c-jun, ATF-2) which can lead to an array of signal transduction cascades including cell death, cell survival and growth. Three JNK isoforms (JNK1, 2, and 3) have been identified, with JNK1 and JNK2 widely expressed in all tissues, whereas JNK3 is selectively expressed in the brain, heart, and testis.[2,3]

In recent studies, JNK-1, often in concert with JNK-2 has been suggested to play a central role in the development of obesity-induced insulin resistance which implies therapeutic inhibition of JNK1 may provide a potential solution in type-2 diabetes mellitus.[4,5] JNK2 has been described in the pathology of autoimmune disorders such as rheumatoid arthritis and asthma, and it also has been implicated to play a role in cancer, as well as in a broad range of diseases with an inflammatory component.[6–10] JNK3 has been shown to mediate neuronal apoptosis which makes inhibiting this isoform a promising therapeutic target for neurodegenerative diseases such as Parkinson's disease, Alzheimer's disease and other CNS disorders.[11–13] Therefore, developing JNK inhibitors as therapeutics has gained considerable interest over the past few years.[14–21]

In this Letter we report a novel series of pan-JNK inhibitors identified in a high-throughput-screening campaign of our in-house sample collection. Compound **1** was identified as an ATP-competitive pan-JNK inhibitor with IC_{50} values 1.0 μM and 0.49 μM versus JNK3 and JNK1, respectively, with no inhibition against p38 (>20 μM). Encouraged by the lead candidates structural simplicity and by its promising degree of selectivity against p38, we initiated lead optimization. Herein we report the synthesis, characterization, and SAR (Structure–Activity-Relationships) of piperazine **1** (see Fig. 1).

The synthesis of analogs of **1** are outlined in Scheme 1. A 1,2-disubstituted-3-nitrobenzene (**2**) was treated with a piperazine or piperidine in toluene and refluxed overnight. The resulting nitrobenzene was reduced to the aniline (**3**) via hydrogenation or $SnCl_2$ and then treated with the various acid chlorides in pyridine to give R^1-derivatized compounds (Tables 1–3, **4**, **6**, and **9**). Further SAR of the piperazine was accomplished by BOC-cleavage and funtionalization to afford analogs of **4** (Table 1).

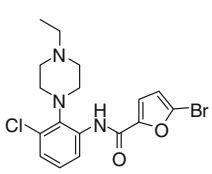

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Figure 1. In-house high-throughput-screening hit (**1**: JNK3 1.0 μM, JNK1 0.49 μM, p38 >20 μM).

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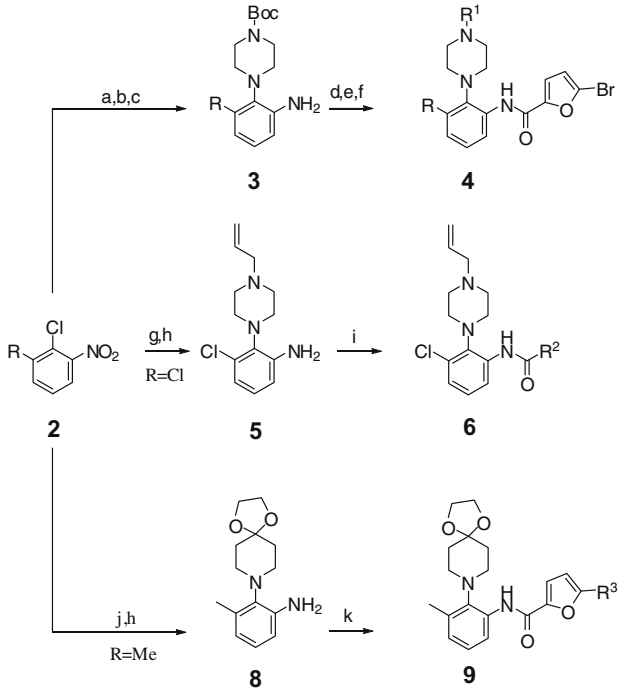


Scheme 1. Reagents and conditions: (a) piperazine, 180 °C, neat (b) Boc_2O (c) H_2, Pt-*O* (d) 5-bromo-furoyl chloride, CH_2Cl_2 (e) trifluoroacetic acid, CH_2Cl_2 (f) R^1Y (Y = leaving group); (g) 1-allylpiperazine, toluene, reflux (h) $SnCl_2$, concd HCl (i) R^2COCl, pyridine, 0 °C (j) 1,4-dioxa-8-azaspiro[4.5]decane, neat (k) 5-R^3-furoyl chloride, CH_2Cl_2, pyridine.

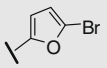
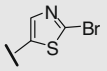
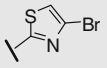
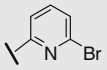
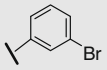
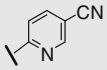
Table 1
N-Acyl-*N*-aryl piperazines

Compd	R	R^1	JNK3[22] IC_{50}[a] (μM)	JNK1[22] IC_{50}[a] (μM)
4a	Cl	H	9.9	4.1
4b	Cl	Me	1.2	0.36
1	Cl	Ethyl	1.1	0.36
4c	Cl	*n*-Pr	0.9	0.63
4d	Cl	*i*-Pr	2.2	1.5
4e	Cl	Allyl	0.33	0.24
4f	Cl	2-Methallyl	0.54	0.40
4g	Cl	Propargyl	0.16	0.14
4h	Cl	Cyclopropyl	0.96	0.18
4i	Cl	Furanylmethyl	0.25	0.27
4j	Cl	Benzyl	1.1	0.88
4k	Cl	Phenethyl	1.4	0.90
4l	Cl	2-Pyridyl	1.0	1.2
4m	Cl	Acetyl	0.81	0.60
4n	Cl	Trifluoroacetyl	>20	>20
4o	Cl	Boc	>20	6.3
4p	Me	Allyl	0.20	0.18
4q	F	Propargyl	0.29	0.11
4r	Br	Allyl	>20	>20
4s	CF_3	Allyl	>20	>20
4t	Ph	Allyl	>20*	>20
4u	NHPh	Allyl	>20*	>20

[a] The values are averages of two or more experiments. All standard deviation ⩽ 20%.
* A number of aryl and heteroaryl substituents tested, all >20 μM.

Compared to the lead structure (**1**), the unprotected piperazine (**4a**) was a 10-fold less potent JNK3 inhibitor while simple alkyl substitutions (**4b–d**) provided little change in potency (Table 1). However, small side chains which contain unsaturation or sp-2 character seemed to provide a small boost in potency (up to six-fold) (**4e–i**). Its unclear if this was the result of a favorable pi-stacking interaction that was no longer available to the sterically larger benzyl or phenethyl analogs. Direct arylation (**4l**) or acylation of the piperazine provided no inhibition advantage (**4m–o**). As for varying the 3-substituent on the phenyl ring, it seemed like small groups were tolerated (Cl, F, Me), but larger groups led to loss of activity (**4r–u**). Like lead hit **1**, all analogs showed no selectivity between JNK3 and JNK1, and if anything, were slightly more potent against JNK1. All compounds also showed no inhibition against p38 (data not shown).

Table 2
Aryl and heterocyclic *N*-acyl amide substitutions

Compound	R^2	JNK3 IC_{50}[a] (μM)	JNK1 IC_{50}[a] (μM)
6a		1.26	0.38
6b		0.31	0.33
6c		1.8	1.8
6d		2.3	nt
6f		5.0	nt
6g		8.9	4.5
6h		5.8	nt
6i		2.0	nt
6j		2.0	1.8
6k		6.4	7.1
6l		>20	>20
6m		>20	>20

[a] Values are means of three experiments; nt, not tested; All standard deviation ⩽ 20%.

SAR of the aniline amide group proved to be equally frustrating (Table 2). We desired to find a replacement for the electron rich furan group with something that might be more metabolically stable, however attempts to modify the 5-bromofuran moiety and maintain potency were difficult.[23] Of all the substitutions examined, the only sub-micromolar inhibitor was 5-bromothiazole **6b**. All others led to substantial losses in potency. Once again, the nature of the interaction, and the special requirement for the bromofuran or thiazole to maintain activity was unclear.

Table 3
Aryl piperidines

Compd	R^3	JNK3 IC_{50}[a] (μM)	JNK1 IC_{50}[a] (μM)
9b	Br	0.06	0.09
9d	Cl	0.08	0.04
9e	F	0.41	0.21
9f	CN	0.21	0.23
9g	Me	0.53	0.33
9h	CHF_2	0.35	0.26
9i	Et	1.0	1.4
9j	Propynyl	0.11	0.11
9k	Bn	4.8	3.2
9l	OMe	0.62	0.45
9m	NHAc	11.0	5.8
9n	NHPh	>20	>20

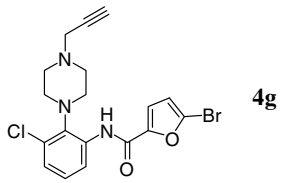
[a] Values are means of three experiments. All standard deviation ⩽ 20%.

Given the finding that 3-methyl substitution was slightly more potency enhancing than 3-chloro (**4p** vs **4e**), future SAR was conducted on the 3-methylsubstituted analogs (Table 3). Additionally, a spiroketal-piperidine was found to be more potency enhancing than the terminally substituted piperazine group. While attempts to replace the bromofuran ring proved challenging, we were able to replace the bromine atom without complete loss of activity. The 5-chlorofuran was the most promising substitution with little affect on potency, however all other substitutions tried, led to a 5–10-fold drop. This drop in potency did not appear to be a size-related phenomena given that both smaller and larger groups are equally less active.

A few compounds from this series were further profiled in a cell-based assay (**4f** IC_{50} = 3.3 μM; **4d** IC_{50} = 2.1 μM) and showed a considerable shift in activity (30–40-fold).[24] Whether the shift arises from the higher ATP concentration in cells or from lack of cell penetration is still under investigation, but this is not an uncommon observation.[25]

To help explain the binding mode of inhibitor to enzyme and to aid in the design of more potent analogs, an X-ray crystal structure of a compound from this class bound to JNK3 was pursued. Fortunately, **4g** provided crystals suitable for X-ray diffraction and the interactions between ligand and enzyme are shown in Figure 2.[26] The inhibitor occupied the ATP binding site, with the bromofuran ring placed deep within the adenosine binding region. The furan was up against the gatekeeper Met 146, but interacting only through van der Waals contacts. The piperazine also formed van der Waals contacts with Ile 70 and Val 78 and to a lesser extent it is interacting in a similar fashion with Val 196 and Leu 206. These are important because they help stabilize the p-loop. The amide oxygen atom in **4g** bound to the backbone hinge (Met 149) in JNK3 and was also close enough to form an electrostatic interaction with the carboxy main chain oxygen of Glu 147. This binding mode is not unlike that described for other structurally unique JNK inhibitors in the literature which benefit not from a strong bidentate interaction with the hinge region of the enzyme, but from the sum of many weak interactions.[27] It may also help to explain why small perturbations in the molecular structure greatly affect inhibition.

In summary, a novel class of piperazine amides were developed from the high-throughput-screening lead **1**. Lead compound optimization produced a series of potent pan-JNK inhibitors inactive against p38 that displayed moderate activity in a JNK cell-based assay. An X-ray crystal structure with JNK3 revealed an unusual binding mode which may be helpful in designing more potent analogs. Synthesis and characterization of these compounds is in progress and will be reported in due course.



4g

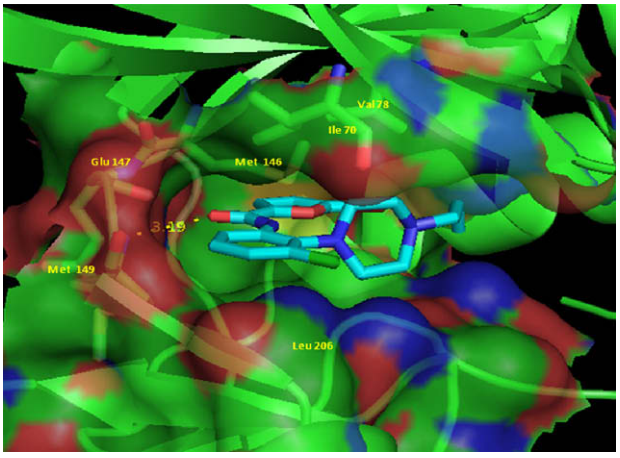


Figure 2. X-ray crystal structure of active site binding **4g** (cyan structure) and JNK3 (green). Critical residues and distance between inhibitor amide oxygen atom and Met 149 amide NH are labeled.

Acknowledgments

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References and notes

1. Barr, R. K.; Bogoyevitch, M. A. *Int. J. Biochem. Cell Biol.* **2001**, *33*, 1047; Weston, Claire R.; Davis, Roger J. *Curr. Opin. Cell Biol.* **2007**, *19*, 142.
2. Gupta, S.; Barrett, T.; Whitmarsh, A. J.; Cavanagh, J.; Sluss, H.; Derijard, B.; Davis, R. J. *EMBO J.* **1996**, *15*, 2760.
3. Mohit, A. A.; Martin, J. H.; Miller, C. A. *Neuron* **1995**, *14*, 67; Carboni, L.; Tacconi, S.; Carletti, R.; Bettini, E.; Ferraguti, F. *Neuroscience* **1997**, *80*, 147.
4. Hirosumi, J.; Tuncman, G.; Chang, L.; Gorgun, C. Z.; Uysal, K. T.; Maeda, K.; Karin, M.; Hotamisligil, G. S. *Nature* **2002**, *402*, 333.
5. Bennet, B. L.; Satoh, Y.; Lewis, A. J. *Curr. Opin. Pharmacol.* **2003**, *3*, 420.
6. Han, Z.; Chang, L.; Yamnishi, Y.; Karin, M.; Firestein, G. S. *Arthritis Rheum.* **2002**, *46*, 818.
7. Wong, W. S. F. *Curr. Opin. Pharmacol.* **2005**, *5*, 264.
8. Pelaia, G.; Cuda, G.; Vatrella, A.; Gallelli, L.; Caraglia, M.; Marra, M.; Abbruzzese, A.; Caputi, M.; Maselli, R.; Costanzo, F. S.; Marsico, S. A. *J. Cell. Physiol.* **2005**, *202*, 642.
9. Blease, K.; Lewis, A.; Raymon, H. K. *Expert Opin. Emerging Drugs* **2003**, *8*, 71.
10. Zhang, H.; Shi, X.; Zhang, Q.-J.; Hampong, M.; Paddon, H.; Wahyuningsih, D.; Pelech, S. *J. Biol. Chem.* **2002**, *277*, 43648.
11. Kyriakis, J. M.; Avruch, J. *Physiol. Rev.* **2001**, *81*, 807.
12. Zhang, G.-Y.; Zhang, Q.-G. *Expert Opin. Invest. Drugs* **2005**, *14*, 1373.
13. Kuan, C.-Y.; Whitmarsh, A. J.; Yang, D. D.; Liao, G.; Schloemer, A. J.; Davis, R. J.; Rakic, P. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 15184.
14. Bennett, B. L.; Sasaki, D. T.; Murray, B. W.; O'Leary, E. C.; Sakata, S. T.; Xu, W.; Leisten, J. C.; Motiwala, A.; Pierce, S.; Satoh, Y.; Bhagwat, S. S.; Manning, A. M.; Anderson, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 13681.
15. Ruckle, T.; Biamonte, M.; Grippi-Vallotton, T.; Arkinstall, S.; Cambet, Y.; Camps, M.; Chabert, C.; Church, D. J.; Halazy, S.; Jiang, X.; Martinou, I.; Nichols, A.; Sauer, W.; Gotteland, J.-P. *J. Med. Chem.* **2004**, *47*, 6921.
16. Graczyk, P. P.; Khan, A.; Bhatia, G. S.; Palmer, V.; Medland, D.; Numata, H.; Oinuma, H.; Catchich, J.; Dunne, A.; Ellis, M.; Smales, C.; Whitfield, J.; Neame, S. J.; Shah, B.; Wilton, D.; Morgan, L.; Patel, T.; Chung, R.; Desmond, H.; Staddon, J. M.; Sato, N.; Inoue, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 4666.

17. Gaillard, P.; Jeanclaude-Etter, I.; Ardissone, V.; Arkinstall, S.; Cambet, Y.; Camps, M.; Chaber, C.; Church, D.; Cirillo, R. A.; Gotteland, J.-P. *J. Med. Chem.* **2005**, *48*, 4596.
18. Swahn, B.-M.; Huerta, F.; Kallin, E.; Malmstrom, J.; Weigelt, T.; Viklund, J.; Womack, P.; Xue, Y.; Phverg, L. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 5095.
19. Stocks, M. J.; Barber, S.; Ford, R.; Leroux, F.; St-Gally, S.; Teague, S.; Xue, Y. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3459.
20. Swahn, B.-M.; Xue, Y.; Arzel, E.; Kallin, E.; Magnus, A.; Plobeck, N.; Viklund, J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1397.
21. For the review of reported JNK inhibitors: LoGrasso, P.; Kamenecka, T. *Mini-Rev. Med. Chem.* **2008**, *8*, 755.
22. Biochemical IC_{50}s for JNKs 1 and 3 were determined using HTRF. Briefly, final assay concentrations of JNK1, JNK3, biotinylated-ATF2 and ATP were 0.1 nM, 0.3 nM, 0.4 nM and 1 μM, respectively. In each assay the phosphor-Thr71-ATF-2 product was detected using a Europium-cryptate labeled anti-phospho-Thr71-ATF-2 antibody. Streptavidin-allophycocyanin-XL was used as the acceptor. A 10-point dose–response curve for each compound was generated in duplicate and data was fit to a four parameter logistic.
23. Most compounds containing the bromofuran moiety had microsome stabilities for rat $\leqslant$5 min.
24. Compounds were assayed for their ability to inhibit phosphorylation of c-jun within the cell by an Enzyme Linked Immunoabsorbent Sandwich Assay (ELISA). In this assay INS-1 β-pancreatic cells were plated in a 96 well tissue culture plate at 3.5×10^4 cells/well (Corning) in a media containing RPMI 1640 (±glutamine (2 mM)) and 10% FBS (Gibco) and incubated overnight at 37 °C in 5% CO_2. An assay plate was prepared by coating a 96 half well plate (Costar) with 50 μl/well p-c-jun capture antibody (Cell Signaling). Cells were incubated with 4 mM Streptozoicin containing various concentrations of potential inhibitor dissolved in DMSO for 3 h at 37 °C in 5% CO_2. After treatment the media was removed and the cells were washed in ice cold PBS. The PBS was removed and the cells were lysed in ice cold lysis buffer (100 ml/well. Cell Signaling) containing 1× protease (Roche) and 1× phosphatase inhibitors (Sigma). Lysates were transferred to the corresponding well of the blocked assay plate, covered tightly and incubated 16hr at 4 °C. The c-jun detection antibody (100× dilution) and the secondary anti mouse coupled HRP (1000× dilution) were purchased from Cell Signaling. Inhibition of signal was quantified using TMB substrate (BioFX Laboratories) and read on a microplate reader at an absorbance of 450 nm. EC_{50} values were determined using a four parameter logistic and a 10-point dilution curve for each of the inhibitors covering four orders of magnitude of inhibitor concentration.
25. Liu, M.; Wang, S.; Clampit, J. E.; Gum, R. J.; Haasch, D. L.; Rondinone, C. M.; Trevillyan, J. M.; Abad-Zapatero, C.; Fry, E. H.; Sham, H. L.; Liu, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 668; Alam, M.; Beevers, R. E.; Ceska, T.; Davenport, R. J.; Dickson, K. M.; Fortunato, M.; Gowers, L.; Haughan, A. F.; James, L. A.; Jones, M. W.; Kinsella, N.; Lowe, C.; Meissner, J. W. G.; Nicolas, A.; Perry, B. G.; Phillips, D. J.; Pitt, W. R.; Platt, A.; Ratcliffe, A. J.; Sharpe, A.; Tait, L. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3463.
26. Crystallographic coordinates of JNK3 complexed with **4g** have been deposited at the Protein Data Bank (www.rcsb.org/) with PDB ID code 3FV8.
27. Angell, R. M.; Atkinson, F. L.; Brown, M. J.; Chuang, T.; Christopher, J. A.; Cichy-Knight, M.; Dunn, A. K.; Hightower, K. E.; Malkakorpi, S.; Musgrave, J. R.; Neu, M.; Rowland, P.; Shea, R. L.; Smith, J. L.; Somers, D. O.; Thomas, S. A.; Thompson, G.; Wang, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1296; Szczepankiewicz, B. G.; Kosogof, C.; Nelson, L. T.; Liu, G.; Liu, B.; Zhao, H.; Serby, M. D.; Xin, Z.; Liu, M.; Gum, R. J.; Haasch, D. L.; Wang, S.; Clampit, J. E.; Johnson, E. F.; Lubben, T. H.; Stashko, M. A.; Olejniczak, E. T.; Sun, C.; Dorwin, S. A.; Haskins, K.; Abad-Zapatero, C.; Fry, E. H.; Hutchins, C. W.; Sham, H. L.; Rondinone, C. M.; Trevillyan, J. M. *J. Med. Chem.* **2006**, *49*, 3563.