Published in final edited form as:

Bioorg Med Chem Lett. 2014 January 1; 24(1): 161–164. doi:10.1016/j.bmcl.2013.11.052.

Synthesis and SAR of novel isoxazoles as potent c-jun N-terminal kinase (JNK) Inhibitors

Yuanjun He, Derek Duckett, Weimin Chen, Yuan Yuan Ling, Michael D. Cameron, Li Lin, Claudia H. Ruiz, Philip V. LoGrasso, Theodore M. Kamenecka, and Marcel Koenig*

Department of Molecular Therapeutics, and Translational Research Institute, The Scripps Research Institute, Scripps Florida, 130 Scripps Way #A2A, Jupiter, FL 33458, USA

Abstract

The design and synthesis of isoxazole **3** is described, a potent JNK inhibitor with two fold selectivity over p38. Optimization of this scaffold led to compounds **27** and **28** which showed greatly improved selectivity over p38 by maintaining the JNK3 potency of compound **3**. Extensive SAR studies will be described as well as preliminary *in vivo* data of the two lead compounds.

As a member of the mitogen-activated protein kinase (MAPK) family, the c-Jun N-terminal kinases (JNKs) regulate the serine/threonine phosphorylation of several transcription factors when they are activated in response of a variety of stress-based stimuli such as cytokines, ultraviolet irradiation, heat shock, fatty acids and osmotic shock.^{1, 2} Three distinct genes (*jnk1*, *jnk2* and *jnk3*) encoding the ten splice variants of JNK have been identified.³ These isoforms differ in their tissue distribution profile and functions, with JNK1 and JNK2 being ubiquitously expressed, whereas JNK3 is expressed predominantly in the brain and at lower levels in the heart and testis.⁴

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.^{5, 6} 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.^{7–11} JNK3 has been shown to play important roles in the brain to mediate neurodegeneration, such as beta amyloid processing, Tau phosphorylation and neuronal apoptosis in Alzheimer's disease, as well as the mediation of neurotoxicity in a rodent model of Parkinson's disease.^{12–14} JNK3 is almost exclusively found in the brain. Identifying potent and selective inhibitors of JNK3 may contribute towards neuroprotection therapies with reduced side effect profiles. JNK Inhibitors may have implications in many therapeutic areas.^{15, 16} Therefore, developing JNK inhibitors as therapeutic agents has gained considerable interest over the past few years.^{15, 17–36}

*Corresponding author. Tel.: +1 561 228 2212. marcelk@scripps.edu (M. Koenig).

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Given the significant body of evidence supporting the role of JNK3 in neurodegenerative disorders, our interest is in discovering potent, selective JNK3 inhibitors with good *in vivo* profiles as potential therapeutics for Parkinson's disease. We previously reported on the synthesis and SAR of 4-phenyl substituted pyrimidines.³¹ Compounds in this class had moderate to good *in vitro* potency, but only modest *in vivo* profiles (rodent pk and brain penetration). Our ongoing research is focused on the discovery of novel chemical entities with improved potency and *in vivo* profiles.

The aminopyrimidine **1** (figure 1, JNK1 IC₅₀ = 26 nM) was previously reported in the literature.³⁷ While 2-amino-4-(hetero)aryl pyrimidine compounds are widely described in the literature, their pyridine equivalents are far less investigated, both synthetically and biologically. We anticipated to discover molecules with promising biological properties and yet patentable by replacing the pyrimidine- by a pyridine core. The very potent dual JNK3/p38 Inhibitor **2** (figure 1) was discovered in our lab,³⁸ with an IC₅₀ = 7 nM for JNK3 and IC₅₀ = 4 nM for p38 in the biochemical assays. While we were intrigued by the potency of compound **2** in the JNK biochemical assay, selectivity over p38 is highly desirable because of potential adverse effects, namely liver toxicity of p38 inhibitors.^{39–41} In order to dial out p38 inhibitory properties, we looked at the crucial binding interactions of these types of molecules with the protein. The aryl-heteroaryl moiety is found in numerous p38 inhibitors. Crystal structures⁴² and molecular modeling^{43, 44} of similar molecules suggest two critical interactions of the pyrazole moiety with the p38 kinase, one being the aryl moiety in the 3-position extending into the deep hydrophobic pocket and the other one the lone pair of the pyrazole nitrogen forming a hydrogen bond to Lys53. We pursued several strategies to achieve selectivity over p38. Our efforts to achieve selectivity in aryl substituted pyrazole analogs are described elsewhere.^{34,45} In this letter we describe modifications on the heterocyclic core. It was shown that replacing the nitrogen with oxygen, a weaker H bond acceptor, will result in reduced p38 potency,⁴⁶ thus we substituted the pyrazole moiety with an isoxazole group. Compound **3** (figure 1, table 1) indeed showed much reduced p38 potency; the JNK3 potency was also reduced, to a lesser extent however. Surprisingly, 4-(isoxazol-3-yl)pyridin-2-amino derivatives are not described in the literature. It is also noteworthy that the isomeric isoxazole, in which the nitrogen and oxygen position were permuted, resulted in a compound with equal JNK3 potency as compared to compound **3** but loss in selectivity over p38. Encouraged by these results, we explored substitutions in the 5 position of the isoxazole group. Indeed we were able to further improve the selectivity over p38 (table 1).⁴⁷

By introducing a methyl group in the 5-position (**6**) we maintained the JNK3 potency but further increased the selectivity to >8 fold. While cyclopropyl (**5**) and mono-methyl amine (**10**) groups are well tolerated, larger groups decreased potency. Interestingly, compound **13** containing a hydroxyl group was found to be exquisitely potent in the JNK3 biochemical assay with approximately 20 fold selectivity over p38 and 30 fold selectivity over JNK1. The stability of compound **13** in human microsoms was very poor however (data not shown). The synthesis of highly substituted isoxazoles via electrophilic cyclisation is reported in the literature^{48–50} and is outlined in scheme 1.

The chloro-pyridines **14** were synthesized in two steps from the commercially available 2-Chloro-4-pyridinecarboxaldehyde and the respective acetylenes using a known procedure⁵¹. This approach allowed for the easy modifications of the 4 and 5 positions of the isoxazole ring and thus efficient exploration of a variety of groups and their effect on the biochemical potency. Alternatively by using the 2-bromopyridine analogs in scheme 1, the reaction sequence of the last two steps may be reversed. Compound **3** as well as the compounds from table 2 were synthesized by using TMS-acetylene, the trimethylsilyl group was cleaved during the Suzuki coupling giving the desired bis-substituted isoxazoles. For a more efficient synthesis of tri-substituted isoxazoles we developed the reaction sequence outlined in scheme 2. Good regioselectivity was achieved. The 5 position may be diversified in the last step by replacing the chloro- group with nucleophiles such as basic amines, alkoxides or nitriles.

Based on the literature, it was concluded that the phenyl group is optimal for p38 potency. As the hydrophobic pocket in JNK is smaller we then went on to explore different substitutions in the 4 position (table 2).

Replacing the aryl with a methyl group (**24**) reduced the JNK potency 10 fold while no inhibition of p38 was observed. By replacing the fluoro phenyl group in compound **3** with an *N*-methyl pyrazole group (**25**) we maintained the JNK3 potency but greatly increased the selectivity over p38. Sterically very hindered pyrazole groups (**29**) reduced JNK potency 10 fold and reduced selectivity over p38. Isomeric pyrazole groups (**31**) reduced JNK3 potency even further while this compound was inactive in the p38 assay. In summary, by introducing substituted or un-substituted pyrazole groups in the 4 position we were able to maintain the good JNK potency but dramatically reduce the p38 potency. Compound **27** was analyzed in a cell based JNK assay ($IC_{50} = 1.5 \mu M$) and *in vivo* (table 3).⁵² This compound had good oral exposure but suffered from a short half live and poor brain penetration. Compound **29**, which has a non-substituted pyrazole moiety, was found to be more potent than the *N*-methyl analog **27** in the biochemical assay ($IC_{50} = 24 \text{ nm}$ vs. 42 nM), but slightly less potent in the cell based JNK assay ($IC_{50} = 1.5 \mu M$ vs. $1.9 \mu M$). The *N*-unsubstituted analog **28** (cell based JNK assay $IC_{50} = 1.8 \mu M$) was found to have poor *in vivo* properties (table 3).

Thus far we focused on modifications on the isoxazole group for potency and selectivity. We have not yet extensively explored the aniline portion of the molecule. Published data of JNK inhibitors containing the 2-aminopyrimidine or 2-aminopyridine motif^{35, 37, 53} suggest that a variety of different groups will be tolerated in the 2-position. Modifications in this part of the molecule may allow to modulate the physicochemical properties and thus potentially to improve the *in vivo* properties and cellular potency.

In summary, we discovered a novel class of isoxazole derivatives as highly potent JNK1/3 inhibitors and greatly improved on the selectivity over the p38 kinase of the lead compound. The cellular potency and *in vivo* properties need to be improved. Through our extensive structure activity (SAR) studies we identified the crucial groups for potency and selectivity. Systematic exploration of diversified 2-anilino groups was not done and may yield compounds maintaining the favorable properties and potentially improving the cellular potency and *in vivo* profile.

Acknowledgments

This work was supported by NIH grant NS057153 awarded to P.L.

References and Notes

1. Barr RK, Bogoyevitch MA. *Int J Biochem Cell Biol.* 2001; 33:1047. [PubMed: 11551821]
2. Weston CR, Davis RJ. *Curr Opin Cell Biol.* 2007; 19:142. [PubMed: 17303404]
3. Gupta S, Barrett T, Whitmarsh AJ, Cavanagh J, Sluss HK, Derijard B, Davis RJ. *EMBO J.* 1996; 15:2760. [PubMed: 8654373]
4. Mohit AA, Martin JH, Miller CA. *Neuron.* 1995; 14:67. [PubMed: 7826642]
5. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. *Nature.* 2002; 420:333. [PubMed: 12447443]
6. Bennett BL, Satoh Y, Lewis AJ. *Curr Opin Pharmacol.* 2003; 3:420. [PubMed: 12901952]
7. Han Z, Chang L, Yamanishi Y, Karin M, Firestein GS. *Arthritis Rheum.* 2002; 46:818. [PubMed: 11920420]
8. Wong WSF. *Curr Opin Pharmacol.* 2005; 5:264. [PubMed: 15907913]
9. Pelaia G, Cuda G, Vatrella A, Gallelli L, Caraglia M, Marra M, Abbruzzese A, Caputi M, Maselli R, Costanzo FS, Marsico SA. *J Cell Physiol.* 2005; 202:642. [PubMed: 15316926]
10. Blease K, Lewis A, Raymon HK. *Expert Opin Emerg Drugs.* 2003; 8:71. [PubMed: 14610913]
11. Zhang H, Shi X, Zhang QJ, Hampong M, Paddon H, Wahyuningsih D, Pelech S. *J Biol Chem.* 2002; 277:43648. [PubMed: 12221076]
12. Crocker CE, Khan S, Cameron MD, Robertson HA, Robertson GS, Lograsso P. *ACS chemical neuroscience.* 2011; 2:207. [PubMed: 21666838]
13. Chambers JW, Pachori A, Howard S, Ganno M, Hansen D Jr, Kamenecka T, Song X, Duckett D, Chen W, Ling YY, Cherry L, Cameron MD, Lin L, Ruiz CH, Lograsso P. *ACS chemical neuroscience.* 2011; 2:198. [PubMed: 21666839]
14. Hunot S, Vila M, Teismann P, Davis RJ, Hirsch EC, Przedborski S, Rakic P, Flavell RA. *Proceedings of the National Academy of Sciences of the United States of America.* 2004; 101:665. [PubMed: 14704277]
15. LoGrasso P, Kamenecka T. *Mini Rev Med Chem.* 2008; 8:755. [PubMed: 18673131]
16. Siddiqui MA, Reddy PA. *J Med Chem.* 2010; 53:3005. [PubMed: 20146479]
17. Bennett BL, Sasaki DT, Murray BW, O'Leary EC, Sakata ST, Xu W, Leisten JC, Motiwala A, Pierce S, Satoh Y, Bhagwat SS, Manning AM, Anderson DW. *Proceedings of the National Academy of Sciences of the United States of America.* 2001; 98:13681. [PubMed: 11717429]
18. Ruckle T, Biamonte M, Grippi-Vallotton T, Arkinstall S, Cambet Y, Camps M, Chabert C, Church DJ, Halazy S, Jiang X, Martinou I, Nichols A, Sauer W, Gotteland JP. *J Med Chem.* 2004; 47:6921. [PubMed: 15615541]
19. Graczyk PP, Khan A, Bhatia GS, Palmer V, Medland D, Numata H, Oinuma H, Catchick J, Dunne A, Ellis M, Smales C, Whitfield J, Neame SJ, Shah B, Wilton D, Morgan L, Patel T, Chung R, Desmond H, Staddon JM, Sato N, Inoue A. *Bioorg Med Chem Lett.* 2005; 15:4666. [PubMed: 16153829]
20. Gaillard P, Jeanclaude-Etter I, Ardisson V, Arkinstall S, Cambet Y, Camps M, Chabert C, Church D, Cirillo R, Gretener D, Halazy S, Nichols A, Szyndralewicz C, Vitte PA, Gotteland JP. *J Med Chem.* 2005; 48:4596. [PubMed: 15999997]
21. Swahn BM, Huerta F, Kallin E, Malmstrom J, Weigelt T, Viklund J, Womack P, Xue Y, Ohberg L. *Bioorg Med Chem Lett.* 2005; 15:5095. [PubMed: 16140012]
22. Stocks MJ, Barber S, Ford R, Leroux F, St-Gallay S, Teague S, Xue Y. *Bioorg Med Chem Lett.* 2005; 15:3459. [PubMed: 15950471]
23. Swahn BM, Xue Y, Arzel E, Kallin E, Magnus A, Plobeck N, Viklund J. *Bioorg Med Chem Lett.* 2006; 16:1397. [PubMed: 16337120]

24. Angell RM, Atkinson FL, Brown MJ, Chuang TT, Christopher JA, Cichy-Knight M, Dunn AK, Hightower KE, Malkakorpi S, Musgrave JR, Neu M, Rowland P, Shea RL, Smith JL, Somers DO, Thomas SA, Thompson G, Wang R. *Bioorg Med Chem Lett*. 2007; 17:1296. [PubMed: 17194588]
25. Alam M, Beevers RE, Ceska T, Davenport RJ, Dickson KM, Fortunato M, Gowers L, Haughan AF, James LA, Jones MW, Kinsella N, Lowe C, Meissner JW, Nicolas AL, Perry BG, Phillips DJ, Pitt WR, Platt A, Ratcliffe AJ, Sharpe A, Tait LJ. *Bioorg Med Chem Lett*. 2007; 17:3463. [PubMed: 17459703]
26. Christopher JA, Atkinson FL, Bax BD, Brown MJ, Champigny AC, Chuang TT, Jones EJ, Mosley JE, Musgrave JR. *Bioorg Med Chem Lett*. 2009; 19:2230. [PubMed: 19303774]
27. Cao J, Gao H, Bemis G, Salituro F, Ledebor M, Harrington E, Wilke S, Taslimi P, Pazhanisamy S, Xie X, Jacobs M, Green J. *Bioorg Med Chem Lett*. 2009; 19:2891. [PubMed: 19361991]
28. Jiang R, Duckett D, Chen W, Habel J, Ling YY, LoGrasso P, Kamenecka TM. *Bioorg Med Chem Lett*. 2007; 17:6378. [PubMed: 17911023]
29. Shin Y, Chen W, Habel J, Duckett D, Ling YY, Koenig M, He Y, Vojkovsky T, LoGrasso P, Kamenecka TM. *Bioorg Med Chem Lett*. 2009; 19:3344. [PubMed: 19433357]
30. Kamenecka T, Habel J, Duckett D, Chen W, Ling YY, Frackowiak B, Jiang R, Shin Y, Song X, LoGrasso P. *J Biol Chem*. 2009; 284:12853. [PubMed: 19261605]
31. Kamenecka T, Jiang R, Song X, Duckett D, Chen W, Ling YY, Habel J, Laughlin JD, Chambers J, Figueroa-Losada M, Cameron MD, Lin L, Ruiz CH, LoGrasso PV. *J Med Chem*. 2010; 53:419. [PubMed: 19947601]
32. Neitz RJ, Konradi AW, Sham HL, Zmolek W, Wong K, Qin A, Lorentzen C, Nakamura D, Quinn KP, Sauer JM, Powell K, Ruslim L, Chereau D, Ren Z, Anderson J, Bard F, Yednock TA, Griswold-Prenner I. *Bioorg Med Chem Lett*. 2011; 21:3726. [PubMed: 21570836]
33. Bowers S, Truong AP, Neitz RJ, Neitzel M, Probst GD, Hom RK, Peterson B, Galemme RA Jr, Konradi AW, Sham HL, Toth G, Pan H, Yao N, Artis DR, Brigham EF, Quinn KP, Sauer JM, Powell K, Ruslim L, Ren Z, Bard F, Yednock TA, Griswold-Prenner I. *Bioorg Med Chem Lett*. 2011; 21:1838. [PubMed: 21316234]
34. Noel R, Shin Y, Song X, He Y, Koenig M, Chen W, Ling YY, Lin L, Ruiz CH, LoGrasso P, Cameron MD, Duckett DR, Kamenecka TM. *Bioorg Med Chem Lett*. 2011; 21:2732. [PubMed: 21185177]
35. He Y, Kamenecka TM, Shin Y, Song X, Jiang R, Noel R, Duckett D, Chen W, Ling YY, Cameron MD, Lin L, Khan S, Koenig M, LoGrasso PV. *Bioorg Med Chem Lett*. 2011; 21:1719. [PubMed: 21316221]
36. Probst GD, Bowers S, Sealy JM, Truong AP, Hom RK, Galemme RA Jr, Konradi AW, Sham HL, Quincy DA, Pan H, Yao N, Lin M, Toth G, Artis DR, Zmolek W, Wong K, Qin A, Lorentzen C, Nakamura DF, Quinn KP, Sauer JM, Powell K, Ruslim L, Wright S, Chereau D, Ren Z, Anderson JP, Bard F, Yednock TA, Griswold-Prenner I. *Bioorg Med Chem Lett*. 2011; 21:315. [PubMed: 21112785]
37. Humphries PS, Lafontaine JA, Agree CS, Alexander D, Chen P, Do QQ, Li LY, Lunney EA, Rajapakse RJ, Siegel K, Timofeevski SL, Wang T, Wilhite DM. *Bioorg Med Chem Lett*. 2009; 19:2099. [PubMed: 19327989]
38. Manuscript in preparation.
39. Lee MR, Dominguez C. *Curr Med Chem*. 2005; 12:2979. [PubMed: 16378500]
40. Dominguez C, Powers DA, Tamayo N. *Curr Opin Drug Discov Devel*. 2005; 8:421.
41. Chung KF. *Chest*. 2011; 139:1470. [PubMed: 21652557]
42. Wang Z, Canagarajah BJ, Boehm JC, Kassisa S, Cobb MH, Young PR, Abdel-Meguid S, Adams JL, Goldsmith EJ. *Structure*. 1998; 6:1117. [PubMed: 9753691]
43. Laufer SA, Wagner GK, Kotschenreuther DA, Albrecht W. *J Med Chem*. 2003; 46:3230. [PubMed: 12852754]
44. Laufer SA, Zimmermann W, Ruff KJ. *J Med Chem*. 2004; 47:6311. [PubMed: 15566301]
45. Manuscript in preparation
46. Laufer SA, Margutti S, Fritz MD. *ChemMedChem*. 2006; 1:197. [PubMed: 16892352]

47. The biochemical and cellular potency as well as the PK properties and brain penetration of all compounds were tested at Scripps Florida. The biochemical IC₅₀s for JNK1 and JNK3 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 mM respectively. Final assay concentrations of p38, biotinylated-ATF2, and ATP were 1 nM, 0.4 nM, and 11.5 mM, respectively. In each assay the phosphor-Thr71-ATF-2 product was detected using 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. See Reference 29 for details of cell-based assays.
48. Waldo JP, Larock RC. *Org Lett*. 2005; 7:5203. [PubMed: 16268538]
49. Waldo JP, Larock RCJ. *Org Chem*. 2007; 72:9643.
50. Waldo JP, Mehta S, Neuenswander B, Lushington GH, Larock RCJ. *Comb Chem*. 2008; 10:658.
51. Saccavini C, Tedeschi C, Maurette L, Sui-Seng C, Zou C, Soleilhavoup M, Vendier L, Chauvin R. *Chemistry*. 2007; 13:4895. [PubMed: 17367097]
52. Rat Pharmacokinetics and Mouse Brain Penetration: Pharmacokinetics of test compounds was assessed in Sprague-Dawley rats (n = 3). Compounds were dosed intravenously at 1 mg/kg and orally by gavage at 2 mg/kg. Blood was taken at eight time points (5, 15, 30 min, 1, 2, 4, 6, 8 h) and collected into EDTA containing tubes and plasma was generated using standard centrifugation techniques. Plasma proteins were precipitated with acetonitrile and drug concentrations were determined by LC-MS/MS. Data was fit by WinNonLin using a noncompartmental model and basic pharmacokinetic parameters including peak plasma concentration (C_{max}), oral bioavailability, exposure (AUC), half-life (t_{1/2}), clearance (CL), and volume of distribution (V_d) were calculated. CNS exposure was evaluated in C57Bl6 mice (n = 3). Compounds were dosed at 10 mg/kg intraperitoneally and after 2 h blood and brain were collected. Plasma was generated and the samples were frozen at -80°C. The plasma and brain were mixed with acetonitrile (1:5 v:v or 1:5 w:v, respectively). The brain sample was sonicated with a probe tip sonicator to break up the tissue, and samples were analyzed for drug levels by LCMS/MS. Plasma drug levels were determined against standards made in plasma and brain levels against standards made in blank brain matrix. All procedures were approved by the Scripps Florida IACUC.
53. Kamenicka T, Jiang R, Song X, Duckett D, Chen W, Ling YY, Habel J, Laughlin JD, Chambers J, Figueroa-Losada M, Cameron MD, Lin L, Ruiz CH, LoGrasso PVJ. *Med Chem*. 2010; 53:419.

Figure 1.
Potent novel JNK inhibitors

Scheme 1.

Reagents and conditions: (a) NH₂OMe·HCl, Na₂CO₃, EtOH, 120° C, 1h; (b) ICl, DCM, rt; (c) Boronic acid, Pd(PPh₃)₄, Na₂CO₃ (aq.), DME, 120° C, 20'; (d) 4-morpholinoaniline, Pd₂(dba)₃, Xantphos, Cs₂CO₃, Dioxane, 90°C, 1–24 h.

Scheme 2.

Reagents and conditions: (a) $\text{NH}_2\text{OME}\cdot\text{HCl}$, Na_2CO_3 , EtOH, 120°C , 1 h; (b) ICl , DCM, rt; (c) 4-morpholinoaniline, $\text{Pd}_2(\text{dba})_3$, Xantphos, Cs_2CO_3 , Dioxane, 90°C , 1–24 h; (d) Boronic acid, $\text{Pd}(\text{PPh}_3)_4$, Na_2CO_3 (aq.), DME, 120°C , 20'; (e) NaOMe/MeOH or $\text{R}'\text{R}''\text{NH}$, TEA, DMF, 80°C , 15 h.

Table 1SAR of 4-Fluorophenyl isoxazoles^a

compound	R	JNK3 IC ₅₀ (μM)	JNK1 IC ₅₀ (μM)	p38 IC ₅₀ (μM)
3	H	0.026	0.160	0.057
4	Ph	>20	nt	nt
5		0.070	nt	0.563
6	CH ₃	0.032	nt	0.265
7	CN	0.126	nt	nt
8		0.523	nt	>20
9	N(Me) ₂	0.366	nt	5.16
10	NHMe	0.027	0.152	0.180
11	NHBn	0.134	nt	0.519
12	NH(CH ₂) ₂ N(CH ₃) ₂	0.216	nt	0.174
13	OH	0.001	0.033	0.020

^aJNK3 biochemical IC₅₀ values are the averages of four or more experiments, and the JNK1 and p38 values are the averages of two or more experiments. All standard deviations are $\leq$ 30%

Table 2

SAR of different modifications in the 4 position^a

compound	R	R'	JNK3 IC ₅₀ (μM)	JNK1 IC ₅₀ (μM)	p38 IC ₅₀ (μM)
24	CH ₃		0.435	>20	>20
25			0.019	nt	19.3
26			0.074	nt	4.15

compound	R	R'	JNK3 IC ₅₀ (μM)	JNK1 IC ₅₀ (μM)	p38 IC ₅₀ (μM)
27			0.052	nt	>20
28			0.016	0.013	5.37
29			0.187	0.098	8.95

compound	R	R'	JNK3 IC ₅₀ (μM)	JNK1 IC ₅₀ (μM)	p38 IC ₅₀ (μM)
30			0.024	0.027	7.57
31			0.587	0.469	>20

^a JNK3 biochemical IC₅₀ values are the averages of four or more experiments, and the JNK1 and p38 values are the averages of two or more experiments. All standard deviations are 30%

Table 3

PK properties of the two lead compounds

compound	<i>a</i> rat pk				<i>b</i> mouse [μM]		
	CL _p (mL/min/kg)	t _{1/2} (h)	V _d (L/kg)	oral AUC (μM ² h)	oral C _{max} (μM)	%F	[plasma] [brain]
27	6	1.5	0.4	5.0	1.5	37	13.4 0.6
28	13	1.8	0.4	0.7	0.3	12	nt nt

a 1 mg/kg IV, 2 mg/kg po;*b* 10 mg/kg ip 2h.