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Bioorganic & Medicinal Chemistry Letters 17 (2007) 668–672
Bioorganic & Medicinal Chemistry Letters

Discovery of a new class of 4-anilinopyrimidines as potent c-Jun N-terminal kinase inhibitors: Synthesis and SAR studies

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Received 29 September 2006; revised 27 October 2006; accepted 30 October 2006
Available online 2 November 2006

Abstract—A new series of 4-anilinopyrimidines has been synthesized and evaluated as JNK1 inhibitors. SAR studies led to the discovery of potent JNK1 inhibitors with good enzymatic activity as well as cellular potency represented by compound **2b**. Kinase selectivity profile and the crystal structure of **2b** are also described.
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The c-Jun N-terminal kinases (JNKs) are members of the mitogen-activated protein kinase (MAPK) family of signaling proteins, and they can be activated in response to various stimuli such as environmental stress, cytokines and fatty acids.[1] Three JNK isoforms (JNK1, 2, and 3) have been identified, with JNK1 and JNK2 widely expressed in tissues, whereas JNK3 is selectively expressed in the brain, heart, and testis.[2] There is growing evidence suggesting that activation of the JNK activity is involved in a number of human disease settings.[3] Therefore, considerable efforts have been directed toward the identification of JNK inhibitors suitable for clinical development.[4]

We were particularly interested in JNK1, an enzyme that was believed to play a key role in linking obesity and insulin resistance.[5] JNK1 disrupts the insulin signaling cascade via phosphorylation of the insulin receptor substrate (IRS-1) at serine[307], which leads to the degradation of IRS-1. JNK1$^{-/-}$ mice show lower fasting blood glucose levels as well as lower insulin concentrations than their wild-type littermates, thus are resistant to diet-induced obesity.[5b] In addition, JNK1 activity is elevated in adipocytes of type 2 diabetic patients.[6] These results imply that inhibitors of JNK1 can potentially increase insulin sensitivity, and can be useful as therapeutics for the treatment of type 2 diabetes.[3]

Recently we reported the discovery of aminopyridine[7] as well as 9-hydroxypyrazoloquinolinones[8] as potent and selective JNK inhibitors. In the course of pursuing additional chemotypes as JNK inhibitors, pyrimidine **1** was identified from a high throughput screening (HTS) (Fig. 1). This compound had an enzymatic IC_{50} of 1.9 μM against JNK1, and it was also selective against two other closely related MAP kinases: p38 and ERK2 (IC_{50}s >100 μM). Recent reports from Merck claimed the use of 4-(4-pyrimidinyl)-5-phenylimidazole derivatives as potent JNK3 inhibitors for targeting neurological disorders,[9] however, their compounds are also active against p38 in contrast to the selectivity observed with our hit compound **1**. Encouraged by the preliminary selectivity profile of compound **1** in addition to its 4-aminopyrimidine structural motif which is amenable

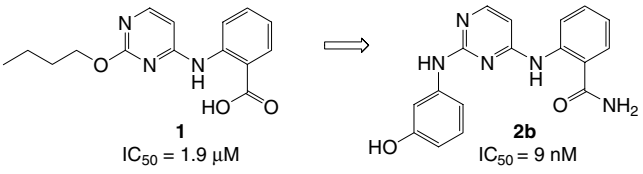

Figure 1. HTL optimization of **1** to potent JNK inhibitor **2b**.

Keywords: JNK inhibitors; Diabetes; Obesity; Kinase.
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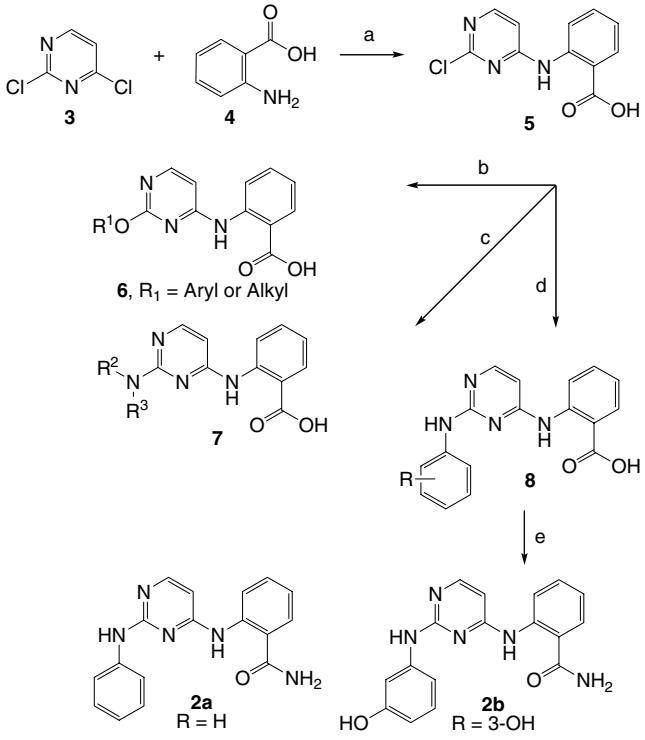
doi:10.1016/j.bmcl.2006.10.093

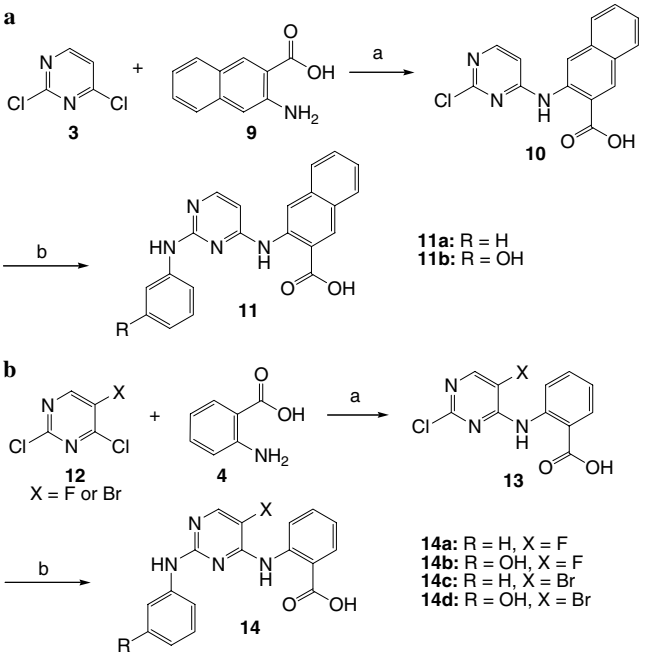
to rapid modification via parallel synthesis, we initiated hit to lead (HTL) chemistry of compound **1**, leading to the discovery of a series of potent JNK1 inhibitors represented by compound **2b**.

The versatile common intermediate **5** was prepared in one step from the substitution reaction between 2,4-dichloropyrimidine **3** and anthranilic acid **4** (Scheme 1).[10] The reaction was regioselective at the 4-position, and can be carried out easily in gram-scale with no purification required. Further reaction (S_NAr) of **5** with an alcohol or phenol in the presence of NaOH, or with a nucleophilic amine provided most of the 2-pyrimidine modification analogs **6**–**8**. Compounds **2a** and **2b** were obtained via TBTU-mediated amide coupling of ammonium chloride with **8**.

Modification of the pyrimidine core or the aromatic ring of the 4-anilino portion was achieved via similar fashion as described for the synthesis of **8**. As outlined in Scheme 2a, analogs **11a** and **11b** were synthesized in a two-step sequence from **3** and 2-amino-3-naphthoic acid (**9**). Similarly, substituting **3** with a halogenated pyrimidine core (**12**) yielded intermediate **13**, where the 2-chloro group may be replaced with an aniline to give compounds **14a**–**d** (Scheme 2b). In the case of 5-bromo-pyrimidine (**12**, X = Br), DMSO was needed as an additional solvent to facilitate the reaction.



Scheme 1. Reagents and conditions: (a) cat. concd HCl, H_2O, 50 °C (1 h), then rt (overnight), 90–95%; (b) phenols or alcohols, NaOH, DMF, microwave 180 °C, 20 min, 50–60%; (c) amines, EtOH, microwave 170 °C, 20 min, 50–60%; (d) anilines, EtOH, microwave 110 °C, 20 min, 60–80%; (e) ammonium chloride, TBTU, DIEA, rt, 6 h, 80–90%.



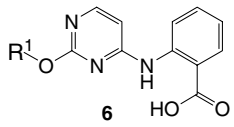
Scheme 2. Reagents and conditions: (a) cat. concd HCl, H_2O (plus DMSO when X = Br in pyrimidine **12**), 50°C (2 h), then rt (2d), 85%; (b) anilines, EtOH, microwave 130 °C, 20–40 min, 55–80%.

During our initial SAR studies, a series of ethers (**6**) bearing different alkyl or aromatic substitutions were prepared and tested against JNK1. As summarized in Table 1, the different straight chain alkyl groups showed only modest variation in potency (**1** and **6a**–**d**), with R^1 = pentyl (**6c**) being optimal with IC_{50} around 1 μM. Few analogs with branching at the β-carbon (**6e**, **6f**) were about 3-fold more active than compound **1**. Phenol ethers provided a 6-fold increase in potency, however, further modification of the phenol ring was detrimental to JNK1 activity. Any additional substitution only resulted in loss of JNK1 activity.[11]

Guided by an NMR-assisted JNK1 active site model (which was later confirmed by X-ray crystallography), we anticipated that the ether portion of the molecule interacted with the hinge region of the enzyme, and replacement of the ether linkage with a mono-substituted amino group should result in an increase in potency with the NH proton acting as a H-bond donor to the carbonyl group within the active site of JNK1. As shown in Table 2, direct replacement of the ether oxygen to NH maintained JNK activity (compare analogs **1** and **7a**). In addition, secondary amines (**7a** and **7c**) were more active than tertiary amines (**7d** and **7e**). To our delight, about a 40-fold improvement in potency was observed when an aniline group was introduced at the 2-position (**8a**). This confirmed our hypothesis that a hydrogen bond donor was needed here to gain JNK1 activity.

Encouraged by the promising results above, we decided to probe the effects of various aniline substitution on JNK1 activity (Table 3). In general, *meta*- or *para*-substituted anilines are more potent than the corresponding ortho analogs. For example, of the

Table 1. SAR summary of JNK inhibitors **1** and **6**

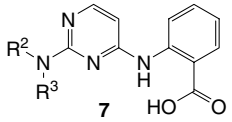


6

Compound	R^1	JNK1 IC_{50}[a] (μM)
1	Bu	1.9
6a	Et	44.7
6b	Pr	2.3
6c	Pentyl	1.1
6d	Hex	>100
6e	*i*-Bu	0.7
6f	CH_2-c-Hex	0.7
6g	Ph	0.3

[a] Values are averages of two or more independent experiments.

Table 2. SAR summary of JNK inhibitors **1**, **7** and **8a**



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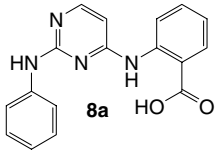


8a

Compound	R^2	R^3	JNK1 IC_{50}[a] (μM)
1	—	—	1.9
7a	Bu	H	2.6
7b	Bn	H	18.2
7c	c-Hex	H	0.4
7d	$-(CH_2)_6-$		15.4
7e	Et	Et	>100
8a	—	—	0.048

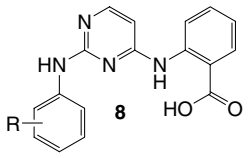
[a] Values are averages of two or more independent experiments.

hydroxyaniline analogs (**8b–8d**), the *meta*-isomer (**8c**) was the most potent among the three, which provided a 2-fold increase in JNK1 activity as compared to the unsubstituted aniline (**8a**). Fluoroanilines (**8e–8g**) exhibited similar potency regardless of the substitution position of the fluoride. All the methyl or trifluoromethyl compounds (**8h–l**) were less potent than the unsubstituted aniline (**8a**), with the *para*-isomer showing somewhat better potency than the *meta*-isomers. An introduction of a nitro (**8m**) or morpholine (**8n**) group at the *para*-position gained slightly in JNK1 activity as well. Heterocycles were also tolerated as exemplified by compounds **15a–c**, which showed similar enzymatic potencies with respect to the reference compound (**8a**).

Next, we synthesized compounds **11** and **14**, aiming to identify additional interactions with the enzyme by targeting the hydrophobic pocket of the protein backbone. As summarized in Table 4, the extra aromatic ring in analogs **11** resulted in a loss of potency (~10-fold). While only a marginal increase in JNK1 activity was seen with compounds **14**, where the 5-hydrogen of the pyrimidine ring was replaced with a halogen, the bromo group could serve as a handle for further optimization at this position.

In order to reach the intracellular target, JNK1 inhibitors need to be cell-permeable. We were concerned that

Table 3. SAR summary of JNK inhibitors (**8** and **15**) with 2-anilino-substitutions



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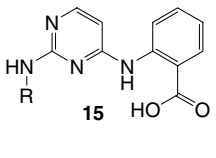
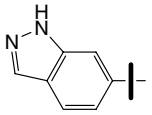
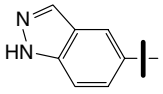
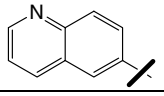
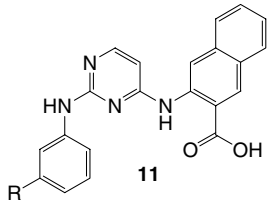


15

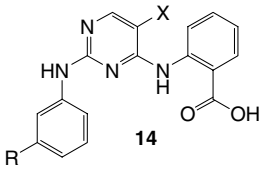
Compound	R	JNK1 IC_{50}[a] (nM)
8a	H	48
8b	2-OH	76
8c	3-OH	25
8d	4-OH	35
8e	2-F	35
8f	3-F	28
8g	4-F	29
8h	3-Me	157
8i	3-F,4-Me	93
8j	2-Me,3-OH	278
8k	3-CF_3	391
8l	4-CF_3	186
8m	4-NO_2	33
8n	4-Morpholine	37
15a		46
15b		55
15c		85

[a] Values are averages of two or more independent experiments.

Table 4. SAR summary for JNK inhibitors **11** and **14**



11



14

Compound	R	X	JNK1 IC_{50}[a] (nM)
11a	H	—	705
11b	OH	—	204
14a	H	F	82
14b	OH	F	21
14c	H	Br	32
14d	OH	Br	20

[a] Values are averages of two or more independent experiments.

the presence of the acid group (which is ionized at physiological pH) in these inhibitors may reduce their capacity to penetrate the cell membrane via passive diffusion. In fact, hit compound **1** showed only 22.3 μM cellular activity in our cell-based c-Jun phosphorylation assay.[12] To address this issue, the acid group was converted to the primary amide (**2**). We were pleased to see that not only the JNK1 enzymatic activity was slightly improved

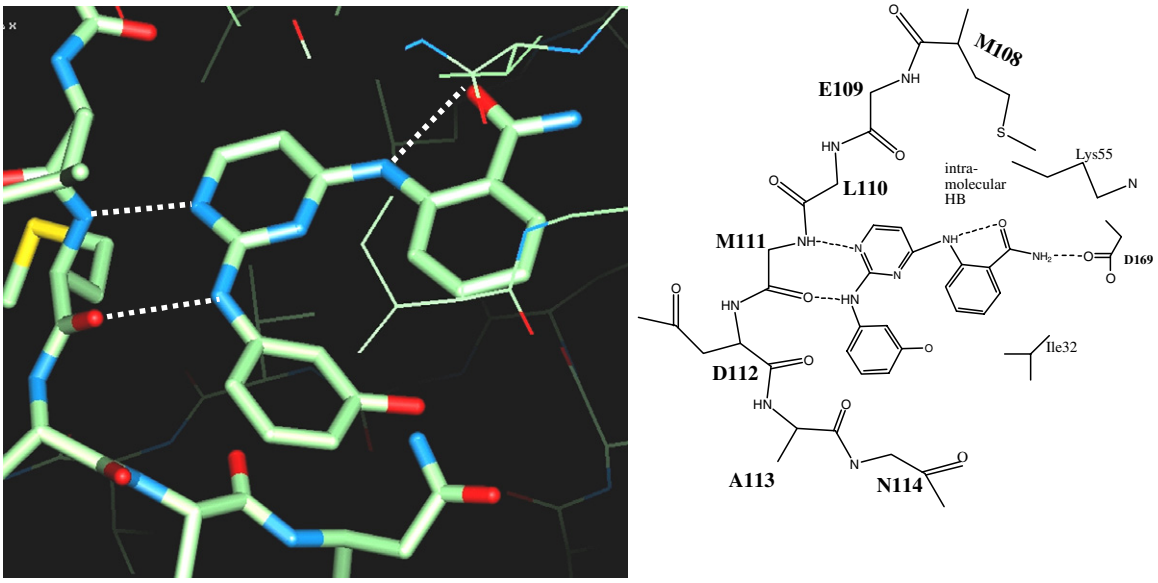


Figure 2. X-ray structure of compound **2b**.

(**2a**: IC_{50} = 34 nM, **2b**: IC_{50} = 9 nM), but also a 20- to 40-fold increase in cellular potency (**2a**: c-Jun IC_{50} = 590 nM, **2b**: c-Jun IC_{50} = 1220 nM) was observed with these compounds.

An X-ray structure of compound **2b** bound into the ATP site of JNK1 has been obtained (Fig. 2).[13] In this structure, two key hydrogen bonds with the pyrimidine N1 acting as a H-bond acceptor and the 2-anilino group serving as a H-bond donor provided the crucial hinge interactions needed for JNK1 activity. This is consistent with the complete loss in potency when the 2-amino group is disubstituted (**7e**, Table 2). An intramolecular H-bond between the carbonyl group and 4-amino group was also evident, which oriented the pyrimidine ring as well as the amide group toward the hydrophobic pocket. Therefore, the extra ring in analogs **11** was actually pointing away from the pocket which we were trying to reach, as a result, about a 10-fold loss in potency was observed. On the other hand, the halogen substitutions of the pyrimidine ring were pointing in the correct direction, which yielded a slight improvement in JNK1 activity. Further extension at this position could potentially identify additional interactions between the ring and the hydrophobic pocket, and thus more potent and possibly selective JNK1 inhibitors may be obtained.

The selectivity data of **2b** against a panel of kinases are listed in Table 5. This compound has some degree of selectivity over quite a few kinases with the exceptions of CHK1, CK2, and PLK. Although compound **2b** shows submicromolar potency against these three kinases, it still appears to exhibit at least 70- to 90-fold greater potency against JNK1 than CHK1, CK2, and PLK.

In summary, we have identified a series of anilinopyrimidines as potent JNK inhibitors with good cellular activity. Since 2-aminopyrimidine is a common pharmacophore for kinase inhibitors, it can be difficult to achieve highly selective inhibitors for this series of compounds. However, there have been examples of selective kinase inhibitors containing the 2-aminopyrimidine structural motifs reported in the literature.[9a,14] Utilizing structure-based design, selectivity was achieved through the modification of the molecule to target the hydrophobic pocket of the enzyme, which is known to contain residues that vary among kinases. Our co-crystal structure of the most potent analog **2b** with JNK1 should guide our future structure-based inhibitor design for obtaining kinase selectivity and perhaps JNK isoform selectivity as well. Additionally, evaluation of ADME/PK properties of these inhibitors and further optimization of them could provide valuable tool compounds for investigating the effects of JNK inhibitors in vivo.

Table 5. Kinase selectivity profile of JNK1 inhibitor **2b**[a]

Kinases	**2b** (μM)	Kinases	**2a** (μM)
JNK1	0.009	PLK	0.73
P38	>50	CK2	0.73
ERK2	25	MEK	6.8
AKT1	15	CDK2	2.7
CHK1	0.82	MK2	>50
PAK4	5.5	COT2	>50

[a] ATP concentrations used were: 1.5 μM for CK2, 5 μM for JNK1, p38, ERK2, PAK4, PLK1, and CHK1, 10 μM for AKT1, MK2, and 100 μM for CDK2, MEK, and COT2.

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