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Design and synthesis of 2′-anilino-4,4′-bipyridines as selective inhibitors of c-Jun N-terminal kinase-3

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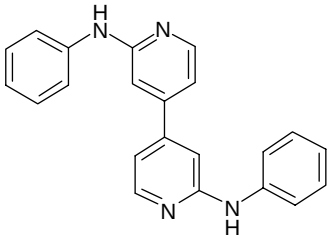
Abstract—The design and synthesis of a new series of c-Jun N-terminal kinase-3 (JNK3) inhibitors with selectivity against JNK1 are reported. The novel series of substituted 2′-anilino-4,4′-bipyridines were designed based on a combination of hits from high throughput screening and X-ray crystal structure information of compounds crystallized into the JNK3 ATP binding active site.

The c-jun N-terminal kinases (JNKs), also called stress-activated protein kinases, belong to the mitogen-activated protein kinase (MAPK) family, which regulate signal transduction in response to environmental stress. Three distinct genes encoding JNKs have been identified (*jnk1*, *jnk2*, and *jnk3*), and at least 10 different splicing isoforms exist in mammalian cells.[1] JNK1 and JNK2 are widely expressed in a variety of tissues, while JNK3 is selectively expressed in the brain and to a lesser extent in the heart and testis.[1,2]

JNK3 knockout mice develop normally and reveal no apparent abnormality but have been shown to be resistant to kainic acid-induced excitotoxicity and associated apoptotic cell death.[3] Mice lacking the *jnk3* gene have also been shown to be protected from brain injury after cerebral ischemia-hypoxia, as well as MPTP (1-methyl-4-phenyl-1,2,4,6-tetrahydropyridine)-induced dopaminergic cell death.[4,5] In contrast, the loss of *jnk1*/*jnk2* genes leads to neuronal tube defects and embryonic lethality.[6,7] For treating neurodegenerative diseases such as stroke, Alzheimer's and Parkinson's diseases it could be beneficial to find JNK3 isoform selective compounds. The three JNK isoforms share more than 90% amino acid sequence identity and the ATP pocket is >98% homologous. It has therefore been a challenge to find JNK3 isoform selective ATP competitive inhibitors.[8] Apart from our recently published paper[9] there is no report of isoform selective compounds even though JNK3 inhibitors have been reported.[8,10,11]

A high throughput screening to find JNK3 inhibitors was conducted, as part of a drug discovery program. Several hits were found and some of these were also co-crystallized with JNK3 and JNK1, and the X-ray crystal structures of the complexes were obtained. The crystal structures of JNK1 and JNK3 have previously been reported,[11–14] but interestingly we found that one of the hits, compound **1** (Fig. 1), from the screening campaign bound into JNK3 and JNK1 in different manners (Fig. 2).[15]



Compound **1**

Figure 1. Hit from high throughput screening.

Keywords: c-Jun N-terminal kinase-3; JNK3 inhibitors; 2′-Anilino-4,4′-bipyridines; Structure based design; Structure–activity relationship.
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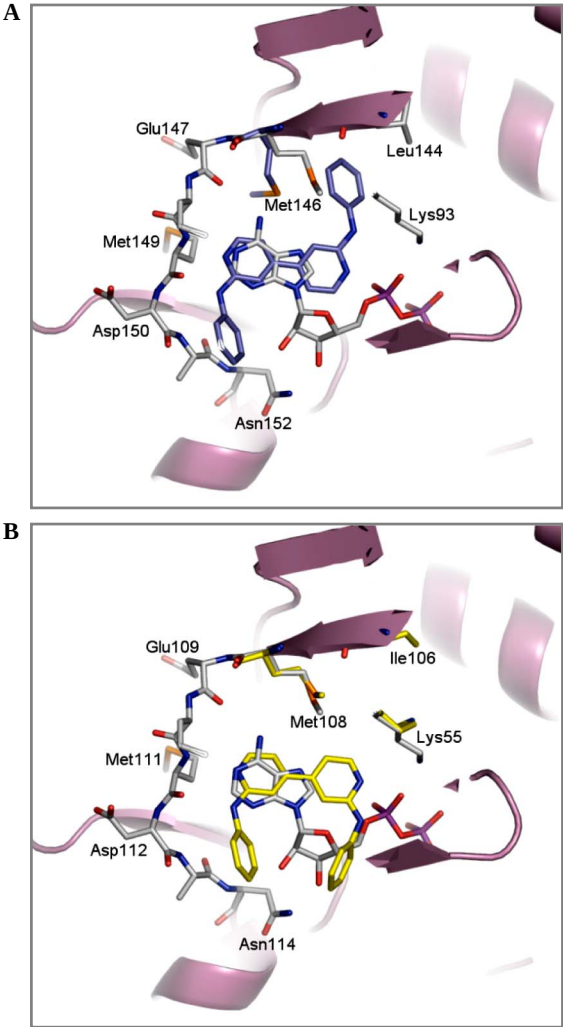
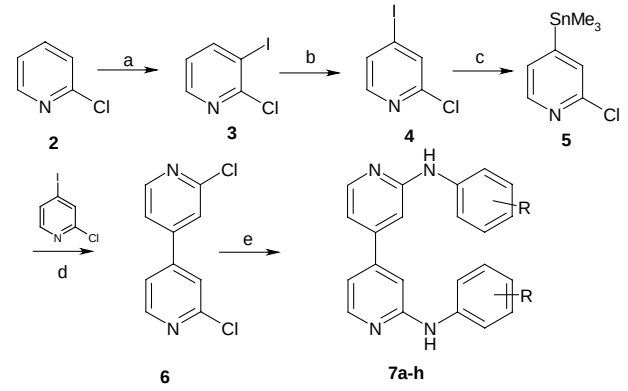


Figure 2. (A)[17,18] Crystal structure of compound **1** in the JNK3 ATP binding site. (B) Compound **1** in the JNK1 ATP binding site.

In JNK3, the 2′-anilino part of molecule **1** binds into the so-called kinase selectivity pocket. The methylthioethyl chain of gatekeeper amino acid Met146 has moved away to accommodate the phenyl group of the ligand in JNK3 compared to the corresponding amino acid Met108 in the JNK1 ATP binding pocket. The pyridine nitrogen and the 2-anilino NH displayed hydrogen bonds to backbone amino acid Met149 in JNK3. Importantly, we could notice a slight increase in activity of compound **1** in JNK3 compared to JNK1 with $IC_{50} = 0.030$ and 0.117 μM, respectively.[16]

We decided to explore if it could be possible to increase the affinity for JNK3 by introducing substituents in the phenyl rings. The initial synthesis of these compounds was performed via coupling of two 2-chloro-4-iodopyridine parts by first synthesizing the tin derivative **5** according to Scheme 1.[19] 2-Chloropyridine **2** was iodinated[20] and then treated with LDA to give a halogen dance[21] to compound **4**. Subsequent halogen-lithium exchange with *n*-BuLi and transmetallation with trimethyl tin chloride gave compound **5** as a non-isolated intermediate. Direct conversion to bipyridine **6** was performed via a palladium catalyzed coupling of intermediate **5** with compound **4**.[22] The 2,2′-dichlorobipyridine was reacted with aniline derivatives under modified Buchwald conditions[23] to yield compounds **7a**–**h**.



Scheme 1. Reagents: (a) *n*-BuLi, I_2, 72%; (b) LDA, 95%; (c) *n*-BuLi, $(CH_3)_3SnCl$; (d) **5**, $(PPh_3)_4Pd$, 53% (c + d); (e) for example, 4-F-aniline, $Pd(OAc)_2$, *t*-BuONa, bis[tri-(*tert*-butyl)phosphine]palladium (0), 59% for R = 4-F.

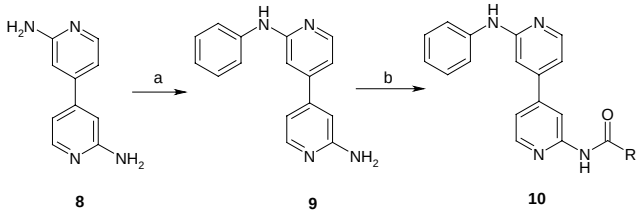
The JNK3 inhibition values for the analogues synthesized according to Scheme 1 are shown in Table 1.

The 4-fluoro and the 2-fluoro analogues **7a** and **7b** were potent JNK3 inhibitors, but these compounds exhibited poor solubility properties, which needed improvement. A search for more soluble bipyridine analogues was conducted by synthesizing compound libraries. We found interesting compounds in a 4,4′-bipyridine-2-amide library **10** synthesized according to Scheme 2.[19] As starting material in these syntheses we used 2,2′-diamino-4,4′-bipyridine **8**, previously described.[24] A palladium catalyzed coupling with iodobenzene[25] yielded the mono 2′-anilino derivative **9** and the amides were subsequently

Table 1. Inhibition results for compounds **7a**–**h** against JNK3

Compound	R	JNK3 IC_{50} (μM)[a]
7a	4-F	0.017
7b	2-F	0.032
7c	2-NH_2	0.108
7d	2-CH_3	0.652
7e	2-OCH_3	0.528
7f	2-OCH_2CH_3	0.693
7g	3-CF_3	>10
7h	4-CF_3	>10

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.



Scheme 2. Reagents: (a) Pd_2dba_3dppf, NaOtBu, PhI, 40%; (b) RCOCl, pyridine, 48% for **10a**.

obtained after treatment with the corresponding acid chlorides in CH_2Cl_2–pyridine.

A large variety of acid chlorides were used, especially many heterocycles and some of the synthesized compounds are shown in Table 2 together with solubility properties and the corresponding IC_{50} values for JNK3 and JNK1.

Compound **10a** (*cis–trans* mixture) looked interesting, since it added new structural features that could be explored further. The corresponding 2′-*p*-fluoroanilino derivative was synthesized followed by separation of the *cis* and *trans* isomers. Interestingly, the *cis* isomer **11** had an IC_{50} value of 15 nM against JNK3 and the *trans* isomer **12** a value of 235 nM. The *cis* isomer **11** was co-crystallized with JNK3 and the X-ray of the complex is shown in Figure 3A.

As can be seen in the crystal structure the methoxy group is in contact with the kinase and will contribute to the binding. In the *trans* derivative **12**, the methoxy group is pointing out toward solvent and does not contribute to binding (not shown).

We also explored SAR of compounds **13** and **14**, which are regioisomers of compound **10d** (Fig. 4). Virtual docking of the three possible regioisomers indicated that the 3-piperidinyl derivative **13** would be the most active and indeed this was the case as is shown in Table 3. The overall kinase selectivity for compound **13** was good as evaluated against a range of kinases and exhibited no significant activity at 10 μM for the majority of the kinases in the panel.[28] Even though we could achieve some selectivity against JNK1 and many other kinases, a disturbing inhibition of the MAP kinase p38α still remained for compound **13**. The 2′-*p*-fluoroanilino derivative **15** was synthesized, because we had noticed that the fluoro substituent generally increased the selectivity against p38α, and indeed this was true also for these analogues.

Table 2. Solubility and JNK inhibition values for compounds **10a–f**

Compound	R	Solubility[26] (μM)	JNK3 IC_{50} (nM)[a]	JNK1 IC_{50} (nM)[a]
7a		<0.1	17	
10a		8	32	106
10b		20	20	451
10c		40	33	261
10d		94	44	219
10e		17	175	780
10f		4	18	42

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.

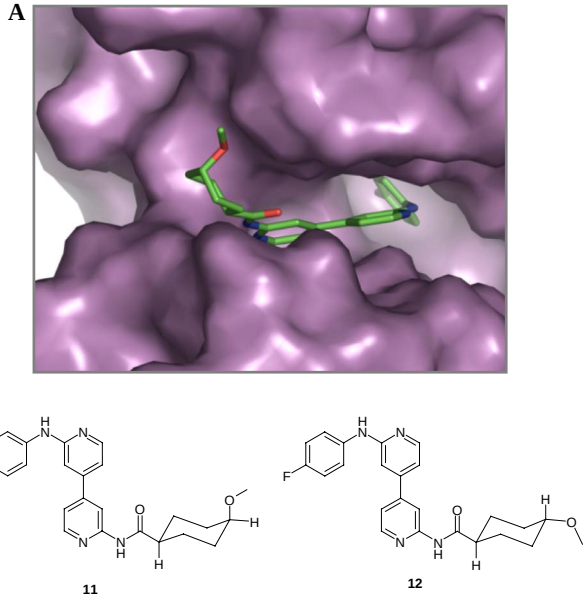


Figure 3. (A) Crystal structure of compound **11** in the JNK3 ATP binding site.[27] (B) Compounds **11** and **12**.

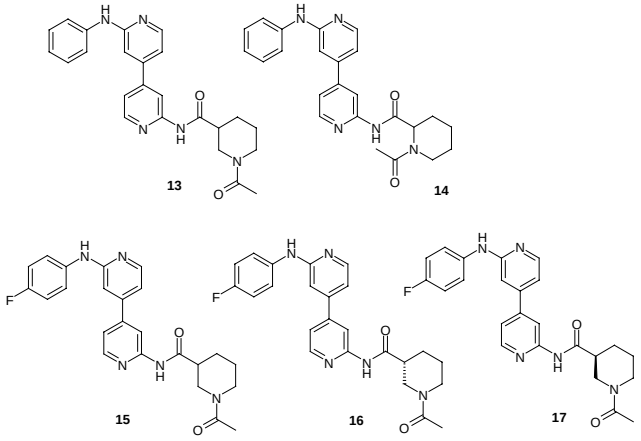


Figure 4. Piperidinyl analogues.

The enantiomers of compound **15**, that is **16** and **17**, were synthesized from chiral starting materials. However, the selectivity against JNK1 and p38α was not improved for these enantiomers as is shown in Table 3.

After resynthesis of some impure compounds in library **10**, by using coupling reagents instead of acid chlorides, we found an interesting tetrahydrofuranyl derivative **18**, solubility 14 μM (Fig. 5). We were pleased to find that the IC_{50} value was 7 nM for JNK3 and 384 nM for JNK1, respectively. In an attempt to further increase the selectivity profile against JNK1 and p38α, the 2′-*p*-fluoroanilino derivative **19** was synthesized and separated by chiral HPLC[30] into the corresponding (+)-enantiomer **20** and (−)-enantiomer **21**. Even though the selectivity against p38α was increased, the selectivity against JNK1 was decreased compared to compound **18** (see Table 3). A good compromise when it came to selectivity was the tetrahydropyranyl derivatives **22** and **23** (see Table 3),

Table 3. Inhibition results for compounds **11**–**20** against JNK3, JNK1, and p38α[29]

Compound	JNK3 IC_{50} (nM)[a]	JNK1 IC_{50} (nM)[a]	p38 IC_{50} (nM)[b]
10d	44	219	n.m.
11	15	88	40
12	235	1280	n.m.
13	9	82	37
14	207	n.m.	n.m.
15	15	85	171
16	5	74	50
17	10	125	162
18	7	384	180
20	3	48	581
21	9	55	421
22	8	122	251
23	6	152	199

n.m., not measured.

[a] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 10\%$.

[b] Values are means of $n \geqslant 2$ determinations, standard deviation $\leqslant \pm 20\%$.

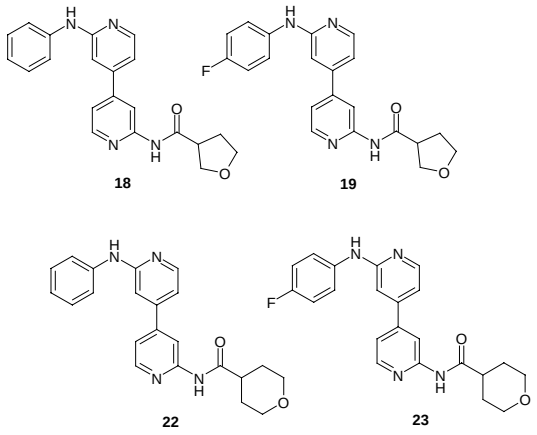


Figure 5. Tetrahydrofuranyl and tetrahydropyranyl analogues.

indeed also the simplest molecules since they contained no chiral center.

Compound **18** displayed a good level of in vitro metabolic stability (rat microsomes Cl_{int} = 12 μL/min/mg) as well as good CaCo2 permeability (29.9×10^{-6} cm/s) and IC_{50} was above 10 μM for the investigated 5 Cyp isoforms. The compound was chosen as the candidate for in vivo PK dosing in rat, and it demonstrated good in vivo characteristics with a bioavailability of 16% (Cl = 30 mL/min/kg; $t_{1/2}$ = 4.7 h po).

In conclusion, a new series of JNK3 selective 2′-anilino-4,4′-bipyridines was described. The binding mode of this new series was confirmed by X-ray crystallographic structures. The large selectivity for these compounds to inhibit JNK3 compared to JNK1 can be attributed to the fact that these compounds bind into the selectivity pocket of JNK3, which has opened up by moving the side chain of Met146 by more than 2 Å. This feature has not been observed when ATP is bound to JNK3, nor when the current series are bound to JNK1. Apparently the induced fit binding into JNK3 was more favorable than for JNK1, and this can probably be explained by the fact that the selectivity pocket has some differences in the amino acid sequence in JNK3 compared to JNK1. Especially Leu144 in JNK3 compared to Ileu106 in JNK1 can play a crucial role in this respect. These 2′-anilino-4,4′-bipyridine derivatives were shown to be potent JNK3 inhibitors demonstrating good metabolic properties in vitro and in vivo as exemplified by compound **18**.

Acknowledgments

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