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Design and synthesis of 6-anilinoindazoles as selective inhibitors of c-Jun N-terminal kinase-3

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Abstract—The structure-based design and synthesis of a new series of c-Jun N-terminal kinase-3 inhibitors with selectivity against JNK1 and p38α is reported. The novel series of substituted 6-anilinoindazoles were designed based on a combination of hits from high throughput screening and X-ray crystal structure information of the 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, whereas JNK3 is selectively expressed in the brain and to a lesser extent in the heart and testis.[1,2]

The 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 lead to neuronal tube defects and embryonic lethality.[6,7] For treating neurodegenerative diseases such as stroke, Alzheimer's, and Parkinson's disease it could, therefore, 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, and so far there is no successful report of such compounds even though some JNK3 inhibitors have been reported.[8,9]

As part of a drug discovery program, a high throughput screening to find JNK3 inhibitors was conducted. Several hits were found and some of these were co-crystallized with JNK3 and the X-rays of the complexes were collected.

The crystal structures of JNK1 and JNK3 have previously been reported,[9–11] but interestingly we found that the known p38 inhibitor, SB203580,[12,13] **1** (Fig. 1) bound into JNK3 in an induced-fit manner.[14]

The methylthioethyl chain of Met 146 had moved to accommodate the *p*-fluorophenyl group of this ligand compared to its position when the ATP analogue ADP **2** was bound (n.b. AMP–PMP and adenylyl imidodiphosphate were used in the crystallization experiment, but only the ADP portion was well-defined in the electron density map). Thus, the *p*-fluorophenyl group entered into what is called the selectivity pocket of kinases. Importantly, we could notice a slight difference in activity for compound **1** in JNK3 compared to JNK1 with IC_{50} = 0.1 and 1.6 μM, respectively.[15]

We concluded that this structural and activity information could be used to increase the selectivity for

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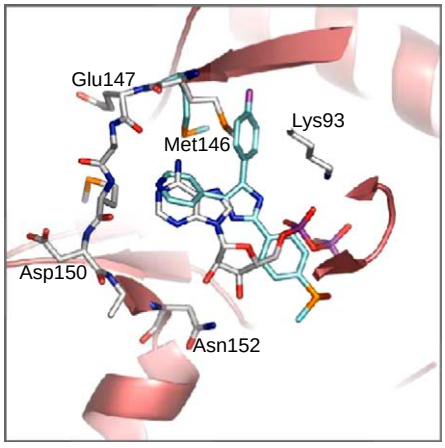


Figure 1. Overlay of X-ray crystal structures of p38 inhibitor **1** (blue) and ADP **2** in the JNK3 ATP binding site.[16,17]

inhibition of JNK3 compared to the inhibition of JNK1. From the high throughput screening campaign, we also found that 3-(4-pyridyl)indazole **3** (Fig. 2) was an inhibitor of JNK3 as well as JNK1.[18] Thus, we anticipated that the 3-arylindazole nucleus would be an interesting scaffold for introducing a substituent that could bind into the selectivity pocket. We predicted by structural comparison that the 6-anilino group in compound **4** should be able to bind in the ATP pocket of JNK3 in an induced-fit manner.

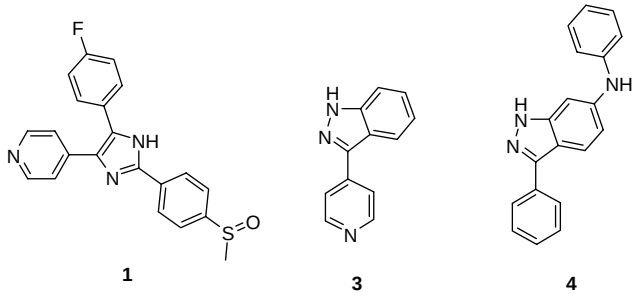


Figure 2. Design of the 6-anilino series of compounds.

The synthesis of compound **4** was conducted as shown in Scheme 1.[19] The starting material was commercially available 6-nitroindazole **5**, which was first iodinated and then N-protected with a Boc group under standard conditions.[20] Compound **6** was then subjected to a Suzuki coupling with a phenyl boronic acid to yield **7**.[20a,21] Thereafter, the nitro group was catalytically reduced and the amine **8** was reacted under Buchwald conditions[22] with an arylbromide to yield **9**. After deprotection of the Boc group, the desired compound **4** was assessed for inhibition of JNK3 and JNK1 as well as co-crystallized with JNK3 to produce the X-ray structure of the complex.

In the X-ray of compound **4a** in the ATP pocket of JNK3 (Fig. 3), it was apparent that our compound bound in an induced-fit manner. The aniline part of the molecule fits

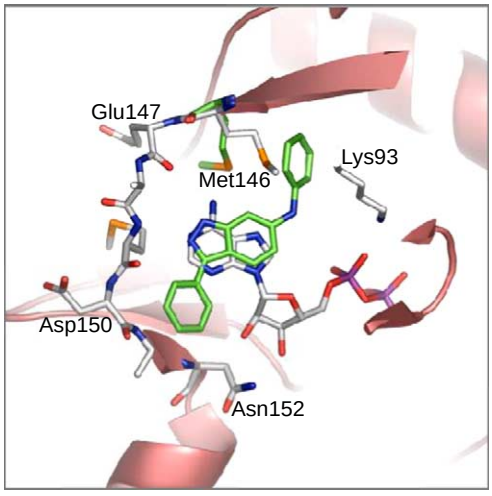
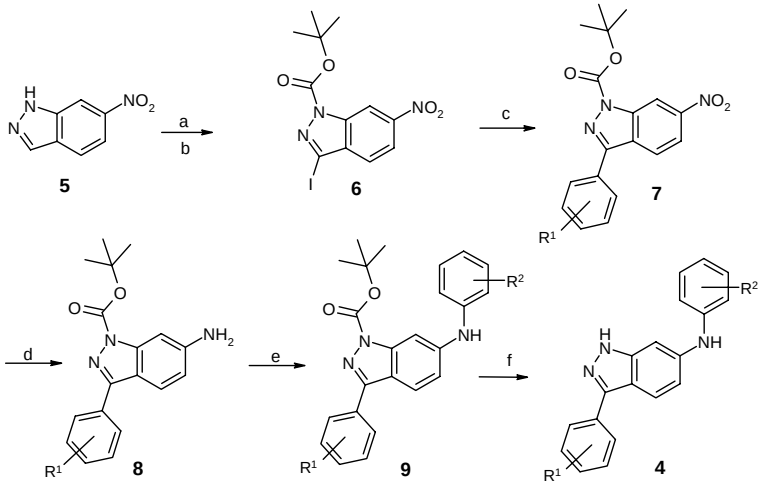


Figure 3. X-ray crystal structure of **4a** (green) in the JNK3 active site overlaid with ADP.



Scheme 1. Reagents: (a) I_2, KOH, DMF, 78%; (b) $(Boc)_2O$, DMAP, Et_3N, CH_3CN/CH_3OH, 82%; (c) Ph-$B(OH)_2$, $PdCl_2(dppf)$, 89%; (d) H_2, PtO_2, 100%; (e) Ph-Br, $Pd(OAc)_2$, S-BINAP, 32%; and (f) HCl, Et_2O/MeOH, 55%. Yields are indicated for compound **4a** in which $R^1 = R^2 = H$.

Table 1. IC_{50} values for compounds **4a–j** against JNK3, JNK1, and p38α[24]

Compound	R^1	R^2	JNK3 IC_{50} (nM)	JNK1 IC_{50} (nM)	p38α IC_{50} (nM)
4a	H	H	48	>10,000	30
4b	H	2-Cl	30	5350	18
4c	H	2-OMe	202	1600	46
4d	4-COOH	2-Cl	32	246	13
4e	4-$CONH(CH_2)_2N(CH_3)_2$	2-Cl	1.9	45	8.7
4f	3-$CONH_2$	2-Cl	3.3	81	3.2
4g	3-COOH	2-Cl	5.3	61	24
4h	3-$CONH(CH_2)_3$-4-morfolinyl	2-Cl	3.4	228	3.7
4i	3-$CONH(CH_2)_2N(CH_3)_3$	2-Cl	21	698	16
4j	3-CONH-4-piperidinyl	2-Cl	1.4	71	4.4

into the selectivity pocket of JNK3 because the side chain of the gatekeeper amino acid Met146 has moved to accommodate the ligand. The indazole nitrogens of compound **4a** displayed hydrogen bonds to backbone amino acids Met149 and Glu147, and the anilino NH hydrogen bond to Lys93 via a water molecule.

Compound **4a** also possessed an excellent selectivity against JNK1 with an IC_{50} value of 0.048 μM for JNK3 and >10 μM for JNK1. These results encouraged us to synthesize more analogues according to Scheme 1 with different R1 and R2 substituents. The corresponding IC_{50} values for JNK3 and JNK1 are shown in Table 1.

The overall kinase selectivity for compounds **4** was good as evaluated against a range of kinases and exhibited no significant activity at 10 μM.[25] Even though we could achieve a large selectivity against JNK1 and many other kinases, a disturbing inhibition of the MAP kinase p38α still remained for compounds **4**. By comparing the crystal structures of compounds **4g** and **4h** in JNK3, we noticed that the carboxylic acid and the amide derivatives bound in different directions (Fig. 4). The 3-carboxylic acid has a hydrogen bond to Asn152, whereas the 3-amide substituents with a sufficiently long chain bearing a basic amine reached out to interact with Asp150. We anticipated that it could be possible to achieve selectivity against p38 by combining these substituents in the same molecule. In p38, the corresponding amino acid to Asn152 is Asp112, that is an acidic amino acid, which would probably reduce the affinity for a negatively charged substituent on the ligand. To test this hypothesis, we decided to synthesize compound **10** which was executed according to Scheme 2.

The SEM protected derivative **11**[26] was found to be a better starting material than the Boc-protected compound **6** because the Boc group was unstable during the subsequent reactions. Compound **11** was treated with 3-amino-5-(methoxycarbonyl)-phenyl boronic acid under Suzuki reaction conditions to yield **12**.[20a,21] The amino group was acylated with 4-dimethylamino butyric acid in the presence of a coupling reagent to afford **13**. Thereafter, the nitro group was reduced and reacted with 1-bromo-2-chlorobenzene under Buchwald reaction conditions to yield **14**.[22] Deprotection of the SEM group with TBAF and then hydrolysis of the methyl ester with LiOH gave the desired compound **10**.

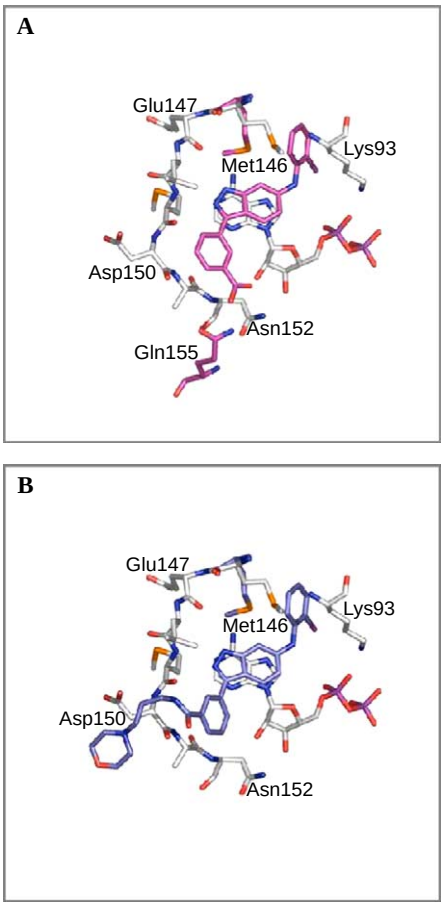
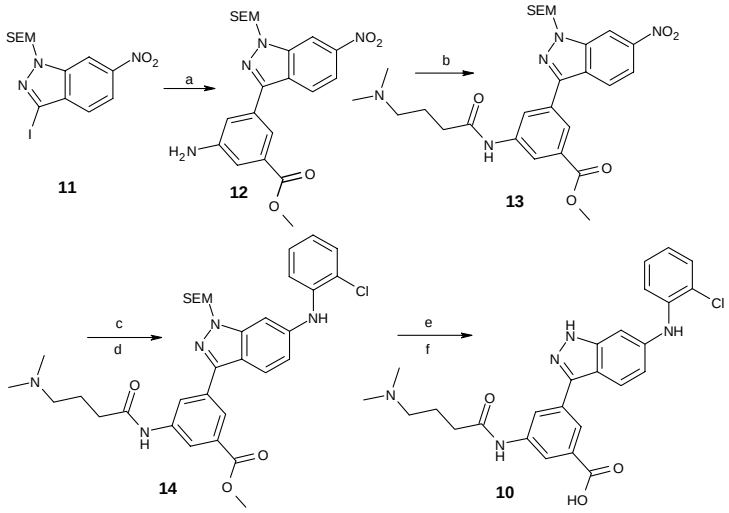


Figure 4. (A) Overlay of X-ray crystal structures of **4g** (magenta) and ADP **2** in the JNK3 ATP binding site. (B) Overlay of X-ray crystal structures of **4h** (purple) and ADP **2** in the JNK3 ATP binding site.

Compound **10** was then examined for inhibitory effect on JNK3, JNK1, and p38. The compound exhibited an excellent selectivity for JNK3 compared to JNK1 and p38 [JNK3 IC_{50} = 3 nM, JNK1 IC_{50} = 101 nM (30×), and p38α IC_{50} = 903 nM (300×)]. Compound **10** was co-crystallized with JNK3 and the X-ray of the complex confirmed the predicted binding mode (Fig. 5A). The carboxylic acid group displayed a hydrogen bond to Asn152 and the basic amine interacted with Asp150.

In conclusion, the structure-based design of a new series of JNK3 selective 6-anilinoindazoles was described.



Scheme 2. Reagents: (a) 3-amino-5-(methoxycarbonyl)phenyl boronic acid, $PdCl_2$(dppf), 54%; (b) 4-dimethylamino butyric acid, HATU, 89% (c) H_2, PtO_2, 99%; (d) 1-bromo-2-chlorobenzene, $Pd(OAc)_2$, S-BINAP, 41%; (e) TBAF, 67%; and (f) LiOH in THF, 72%.

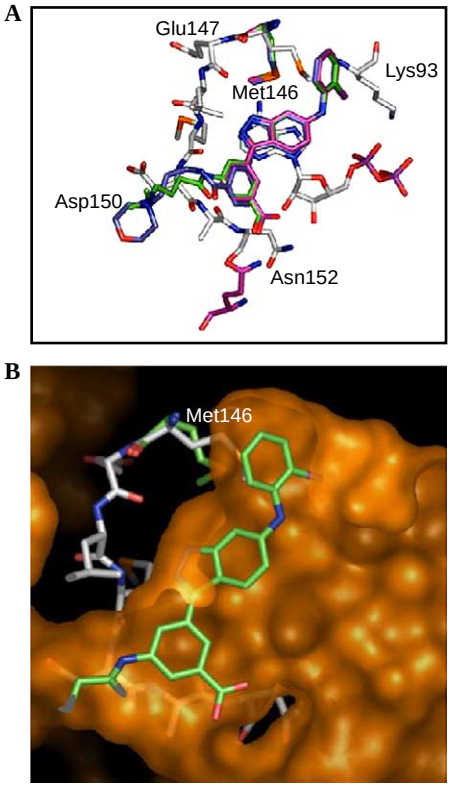


Figure 5. (A) X-ray crystal structure of indazole **10** (green) in JNK3 active site overlaid on ADP **2**, **4g** (magenta) and **4h** (purple). (B) Cutaway of X-ray crystal structure of **10** in JNK3.[23] The PDB deposition code is 2B1P for this structure.

The compounds were synthesized and the binding modes of these new compounds were confirmed by X-ray crystallographic structures. The large selectivity for these compounds to inhibit JNK3 compared to JNK1 could be attributed to the fact that these compounds bound into the selectivity pocket of JNK3. Apparently, the induced-fit binding into JNK3 was more favorable for JNK3 than for JNK1. The amino acid sequence in the selectivity pocket of JNK3 differs somewhat from JNK1 and is probably the explanation for the difference in inhibitory activity of these ligands in JNK3 and JNK1.

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