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Structure-driven HtL: Design and synthesis of novel aminoindazole inhibitors of c-Jun N-terminal kinase activity

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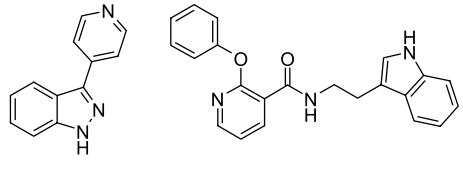
Abstract—The design and synthesis of a new series of c-Jun N-terminal kinase inhibitors are reported. The novel series of substituted amino indazoles were designed based on a combination of hits from high-throughput screening and X-ray crystal structure information of the compounds crystallised into the JNK-1 ATP binding site.

Cell surface receptors play a fundamental role by which cells receive information from extracellular signals. These receptors transmit information into the cell where it is propagated by activation or suppression of biochemical pathways. Protein kinases and phosphatases are important components of such intracellular signalling pathways as they allow the information to be cascaded to numerous effector molecules as well as giving amplification of the extracellular signal. The mitogen-activated protein (MAP) kinase signalling pathways are activated by engagement of a number of cell surface receptors. One of these pathways, the JNK pathway (named after one of the key enzymes in the pathway, c-Jun N-terminal kinase or JNK), is activated specifically by stress or pro-inflammatory cytokines.[1] Members of the JNK family (JNK-1, 2 and 3) act as an integration point for multiple intracellular biochemical signals governing a wide variety of cellular processes such as proliferation,[2] apoptosis,[3] migration[4] and transcriptional regulation.[5] Activation of the JNK pathway has been documented in a number of disease settings, suggesting that specific inhibition of JNK activity could provide an effective therapy for a variety of conditions.[6,7]

Compounds **1**[8] and **2** (Fig. 1) were identified from high-throughput screening and possessed promising potency[9] as well as attractive structural features amenable to optimisation by rapid parallel synthesis.

A series of synthesis/screening cycles based around compound **2** resulted in new lead series of compounds **3**–**10** (Fig. 2), which demonstrated good potency against JNK-1 (Table 1).

An X-ray crystal structure of **4** bound into the ATP binding site of JNK-1 was obtained.[10,11] In the crystal structure (Fig. 3), the phenoxy group binds in the pocket occupied by the ribose of the competitive binder ATP (n.b. AMP-PNA, adenylyl imidodiphosphate, was used in the crystallisation experiment, but only the ADP portion was well defined in the electron density map). The amide present in **4** is acting as a H-bond acceptor making a key interaction with the hinge-region through



1 JNK-1 pIC_{50} 5.8 **2** JNK-1 pIC_{50} 5.7

Figure 1. Initial hits from high-throughput screening.

Keywords: Aminoindazole; c-Jun N-terminal kinase; Inhibitors; Bioavailable.
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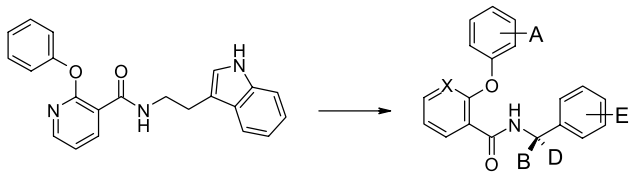


Figure 2. Result of synthesis/screening cycles—identification of a new lead series **3**–**10**, the aryloxy pyridines.

Met111 (2.8 Å). Interestingly, unlike many other kinase inhibitors mimicking the adenine of ATP, compound **4** does not possess a H-bond donor to interact with the hinge-region through Glu109 (Fig. 4).[12]

Unfortunately, structurally similar compounds of the class **3**–**10** were known and exemplified in the patent literature[14] against other biological targets, so a new chemical lead series was required. The X-ray crystal structure of **1** crystallised into JNK-1 was solved and in this case compound **1** makes a donor/acceptor interaction with both the methionine and glutamic acid in the ATP binding site. An overlay of **1** with the X-ray crystal structure of **4** (Fig. 5) suggested that amino indazoles of the type shown in Figure 6 would represent a series of compounds to explore, as the H-bond donating group within the amino indazole could interact with the glutamic acid residue in the hinge-region leading to more highly bound inhibitors.

A series of aminoindazoles was prepared and their JNK-1 activity is shown in Table 2.

An analysis of the X-ray crystal structure of **23** in JNK-1 (Fig. 7) shows the formation of an internal hydrogen bond between the benzylic nitrogen and the phenyl ether of the aminoindazole (2.9 Å) as well as giving evidence of strong H-bond donor/acceptor interactions with Glu109 (2.9 Å) and Met111 (3.1 Å). Further analysis of the structural requirements for binding showed that changing the template from indazole to azaindazole (**11** vs **22**; **14** vs **23**) resulted in a ~10-fold increase in potency against the isolated enzyme. This increase might, in part, be accounted for by considering the X-ray crystal structure, which shows that the 5-position nitrogen of the azaindazole is hydrogen bonding to a water molecule. This water is not observed in all JNK-1 crystal structures, but is positioned close to the well-conserved Lys55 along with a small cluster of other water molecules, suggesting that the local environment in the plane of the aromatic ring is hydrophilic. The azaindazole template can make a good hydrogen bond to the water molecule, an interaction which is absent in the indazole template. A second rational for the observed increase in potency might be due to differences in the electronics of

Table 1. Inhibition results for compounds **3**–**10** against JNK-1

Compound	X	A	B	D	E	Activity[a] pIC_{50} (±0.2)
3	C	H	H	H	2-Cl	5.4
4	N	H	H	H	2-Cl	5.5
5	N	H	H	H	H	5.4
6	N	H	Me	H	H	i.a[b]
7	N	H	H	Me	H	5.2
8	N	H	H	H	2,5-diOMe	7.0
9	N	2-F	H	H	2-Cl	5.8
10	N	4-OMe	H	H	2-Cl	5.4

[a] Activity reported against the isolated JNK-1 enzyme.
[b] <40% inhibition at 100 μM.

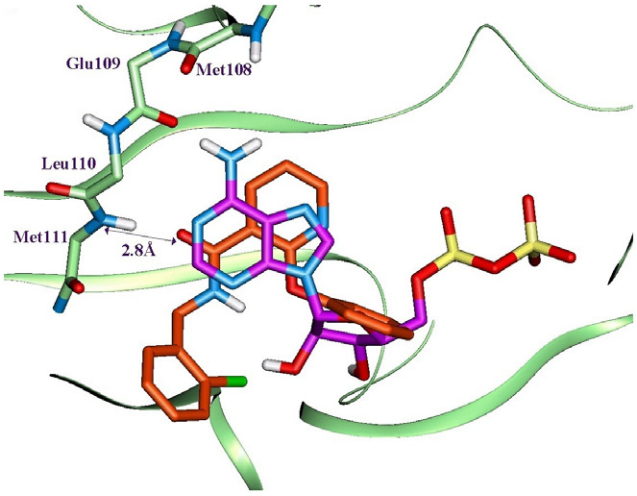


Figure 3. X-ray crystal structure of **4** in the JNK-1 active site overlaid with ADP.[13]

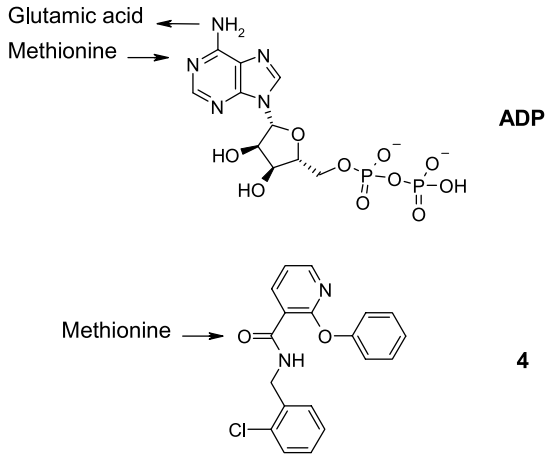


Figure 4. Schematic representation of overlay of **4** with ADP showing key H-bond interactions.

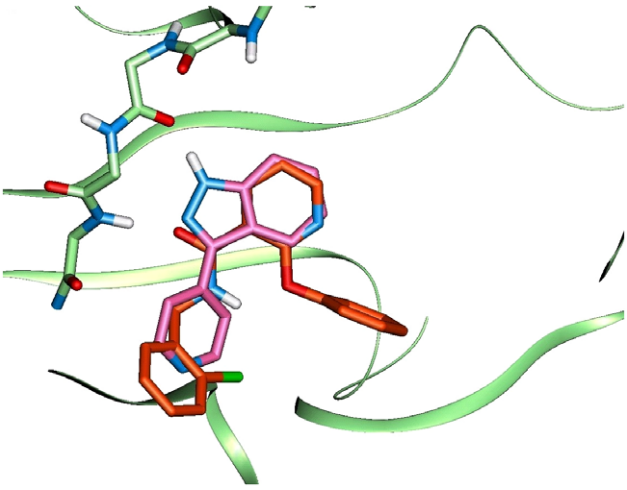


Figure 5. Overlay of X-ray crystal structures of **1** and **4** in the JNK-1 ATP binding site.[15]

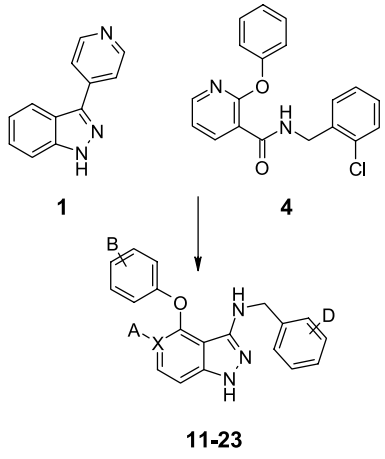


Figure 6. Rational design of the aminoindazole series of compounds.

the pyrazole hinge region between the azaindazole and the indazole templates. This is enforced by calculations showing the N–H at the 1-position of the azaindazole ring system is a better H-bond donor to Glu109 than the corresponding indazole.

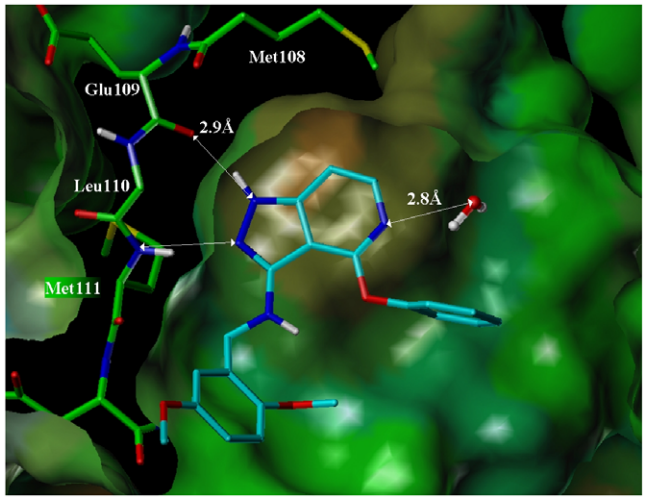


Figure 7. Cutaway of X-ray crystal structure of **23** in the JNK-1 ATP binding site showing the key interactions with Glu109 and Met111.[16]

Compound **23** was found to be metabolically unstable in vitro in both rat and human (rat heps Cl_{int} = 43 μL/min/1×10^6 cells; human mics. Cl_{int} = 74 μL/min/mg). However, compound **22** displayed a good level of in vitro metabolic stability (rat heps Cl_{int} = 10 μL/min/1×10^6 cells; human mics. Cl_{int} = 12 μL/min/mg) and was chosen as the candidate for in vivo PK dosing. The compound demonstrated good in vivo characteristics with good bioavailability (Cl = 34 mL/min/kg; $t_{1/2}$ = 0.7 h; F = 50%).

The synthesis of the aminoindazoles was achieved by known chemistry[17] from commercially available 2-phenoxy-6-fluorobenzonitrile (Scheme 1).

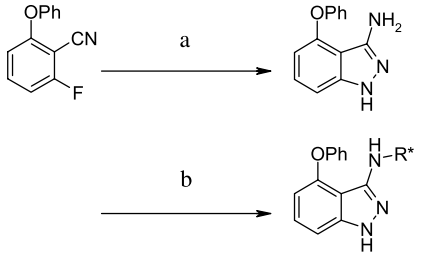
The synthesis of compounds **22** and **23** required the preparation of 4-methoxy-3-cyano-2-chloropyridine[18] (Scheme 2).

In summary, a new series of aminoindazole inhibitors of c-Jun N-terminal kinase activity has been developed from a structure-based drug design programme initiated from a high-throughput screening/synthesis campaign. The initial hit series proved to be reasonably active inhibitors, and X-ray structural information obtained

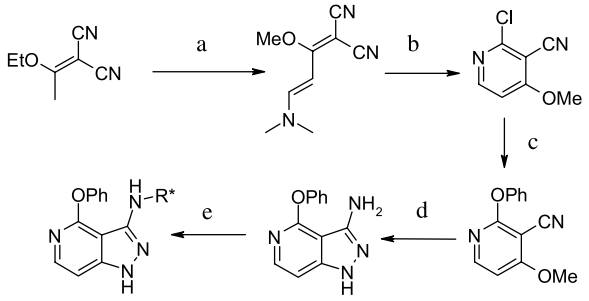
Table 2. Inhibition results for compounds **11**–**23**

Compound	X	A	B	D	Activity[a] pIC_{50} (±0.2)
11	C	H	H	4-NHAc	6.1
12	C	H	H	2,5-diCl	6.1
13	C	H	H	3-Cl	5.9
14	C	H	H	2,5-diOMe	6.9
15	C	H	4-F	4-NHAc	6.3
16	C	H	4-F	2,5-diOMe	6.7
17	C	H	4-SO_2Me	4-NHAc	6.1
18	C	H	4-SO_2Me	2,5-diOMe	6.4
19	C	NO_2	H	2,5-diOMe	6.1
20	C	NO_2	H	2-Cl	5.8
21	C	NH_2	H	2-Cl	5.3
22	N		H	4-NHAc	6.8
23	N		H	2,5-diOMe	7.8

[a] Activity reported against the isolated JNK-1 enzyme.



Scheme 1. Reagents: (a) hydrazine hydrate, ethanol, (b) aldehyde R*CHO, sodium triacetoxyborohydride, methanol, acetic acid.



Scheme 2. Reagents: (a) dimethylformamide dimethyl acetal, methanol, (b) dry HCl, methanol, (c) phenol, cesium carbonate, (d) hydrazine hydrate, ethanol, (e) aldehyde R*CHO, sodium triacetoxyborohydride, methanol, acetic acid.

of these compounds in the active site of JNK-1 eventually afforded the aminoindazole series. These were shown to be potent inhibitors of JNK-1, demonstrating good in vitro pharmacokinetics and good bioavailability when dosed in rats.

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