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Structure-based design and parallel synthesis of *N*-benzyl isatin oximes as JNK3 MAP kinase inhibitors

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ABSTRACT

A series of N-benzylated isatin oximes were developed as inhibitors of the mitogen-activated kinase, JNK3. X-ray crystallographic structures aided in the design and synthesis of novel, selective compounds, that inhibit JNK3, but not p38 MAP kinase and provided key insights into understanding the behavior of gatekeeper residue methionine-146 in determining target selectivity for this series.

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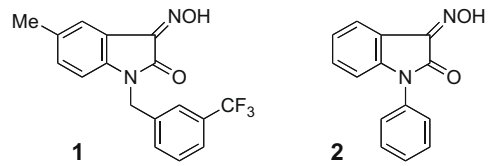
The c-Jun N-terminal kinases (JNKs, also known as stress activated protein kinases, SAPKs) are members of the mitogen activated protein (MAP) kinase family.[1] The MAP kinases are a family of structurally-related serine/threonine kinases that also includes p38 and the extracellular-signal regulated kinases (ERKs). The JNK subfamily consists of ten known isoforms encoded by three genes, jnk-1, jnk-2, and jnk-3, the latter being expressed primarily in the brain and at low levels in the testes and kidney. JNK3 knockout mice were developmentally normal, yet show decreased neuronal death in response to kainic acid[2] and MPTP induced cell death,[3] as well as resistance to hypoxic ischemia.[4] Thus inhibition of JNK3 offers potential for the treatment of neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, epilepsy and seizures, as well as for the treatment of ischemic brain injury.

A number of JNK3 inhibitors have been reported in the literature, notably including SP-600125,[5] and CEP-1347,[6] the first JNK pathway inhibitor to enter clinical development. Recently a number of publications have described a variety of molecules that exhibit different levels of JNK inhibition and selectivities[7–13] (Fig. 1).

In this Letter, we present optimization of an isatin oxime screening hit through application of a combination of parallel synthesis and X-ray crystallography to develop potent and selective JNK3 inhibitors.

To identify novel inhibitors of JNK3 we performed a screen of our corporate compound collection and identified isatin oxime compound **1** as an ATP-competitive JNK3 inhibitor, with moderate potency for JNK3 (K_i = 2.7 μM) and clear selectivity over the MAP kinase family members ERK2 (>30 μM) and p38α (>100 μM).



It was quickly established that O-methylation and N-dealkylation abolished activity, indicating the importance of the free hydroxyl and the *N*-benzyl groups for enzyme inhibition. In addition, the *N*-phenyl compound **2** was also significantly less active (JNK3 K_i > 30 μM), than compound **1**.

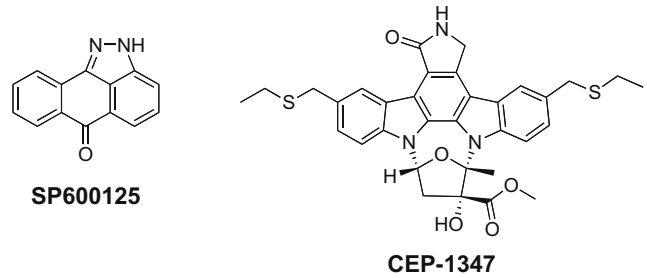


Figure 1. JNK inhibitors SP600125 and CEP-1347.

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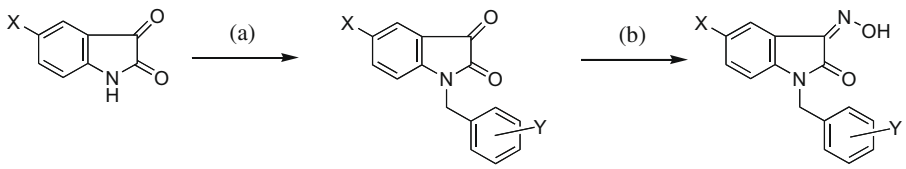
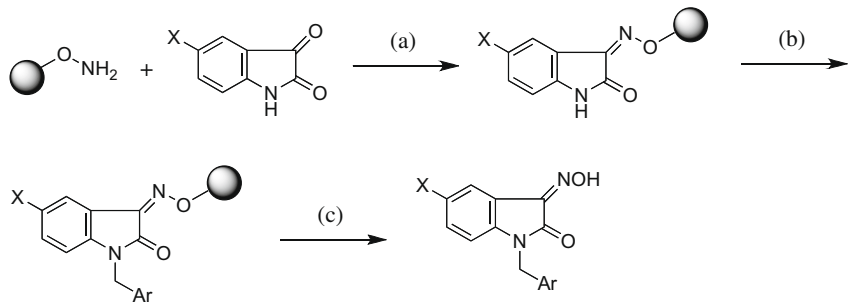


Scheme 1. Preparation of *N*-benzylisatin oximes. Reagents and conditions: (a) $ArCH_2Cl$ or $ArCH_2Br$ (1.1 equiv), K_2CO_3 (4 equiv), DMF, 60 °C, 4 h; (b) 1 M $NH_2OH{\cdot}HCl$ in DMF (4 equiv), rt, 1 h (95% over two steps, where X = F, Y = H).



Scheme 2. Solid-phase synthesis of isatin oximes. Reagents and conditions: (a) DMF, pyridine, 16 h; (b) $ArCH_2Cl$ or $ArCH_2Br$, DBU, DMF, 35 °C, 48–72 h; (c) TFA, CH_2Cl_2, 1 h. Yields 12–77% overall from hydroxylamine resin.

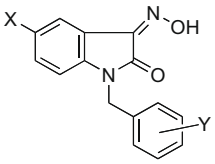
These discoveries led us to investigate further the synthesis and inhibitory activities of *N*-benzyl isatin oximes. Initial work to develop SAR employed two synthetic approaches, starting with the commercially available isatins. In the first approach (Scheme 1) a solution-phase method was developed that was amenable to parallel synthesis. In this scheme the reaction conditions were selected such that the isatin in DMF was added to excess K_2CO_3, followed by addition of benzyl bromides or chlorides and the reactions left to mix at 60 °C for 6 h. Addition of excess hydroxylamine hydrochloride converted the isatin to the corresponding oxime, and any remaining alkylating agent was sequestered by the hydroxylamine to produce a more water-soluble benzyl hydroxylamine, thereby facilitating a simple aqueous work-up.

In the second approach (Scheme 2) a solid-phase method was developed using the TFA-cleavable hydroxylamine resin described by Richter and Desai.[14] *O*-*p*-Alkoxybenzyl hydroxylamine resin was treated with substituted isatins in a mixture of DMF and pyridine and shaken at ambient temperature overnight. Thorough washing of the resin (DMF ×2, MeOH ×2, CH_2Cl_2 until no color was observed), followed by cleavage of a small sample with 50% TFA in CH_2Cl_2 released the isatin oxime together with a small amount of byproduct arising from cleavage of the linker from the resin. This side reaction has been previously noted with this hydroxylamine Wang resin.[15] The resin-bound isatin oxime was N-alkylated by treatment with alkyl chloride or bromide (2 equiv) in DMF in the presence of DBU (2 equiv). After 48–72 h at 35 °C the resin was washed with DMF, MeOH, and CH_2Cl_2. Alkylation at elevated temperatures (e.g., 80 °C) resulted in shorter reaction times, but poorer quality product. N-Alkylated isatin oximes were released from the resin with 50% TFA in CH_2Cl_2 and products were purified by preparative HPLC.

Table 1 illustrates data for the N-benzylated isatin oxime derivatives prepared from commercially available isatins. It is evident that the SAR for the isatin substituents (X) was relatively flat. However the commercially available isatins represent very limited structural diversity. More significant variation in enzyme potency was observed from the N-substituent. In particular the 3,5-dimethoxybenzyl (compounds **15**, **24**) and the 6-fluoro-4*H*-benzo[1,3]-dioxin-8-ylmethyl (compounds **25**–**27**) substituents conferred the most potency.

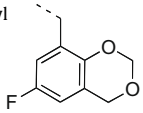
At this point we were able to obtain an X-ray crystallographic structure of compound **25** bound to the ATP-site of JNK3 (Fig. 2) that revealed the key structural elements needed for binding.[17] The isatin carbonyl oxygen is H-bonded to the NH of Met149 in the hinge region that links the two kinase subdomains, with the oxime hydroxyl H-bonded to the backbone carbonyl of residue

Table 1
JNK3 inhibition data for 5-substituted isatin derivatives



Compd	X	Y	JNK3 K_i (μM)[16]
3	H	H	>10
4	H	2-F	8.5
5	H	3-F	8.1
6	H	4-F	8.3
7	H	2-NO_2	>10
8	H	3-NO_2	0.94
9	H	4-NO_2	7.0
10	H	3-Cl	5
11	H	4-Cl	6.7
12	H	3-Me	6.8
13	H	4-Me	9.9
14	H	3-CF_3	5.2
15	H	3,5-$(OMe)_2$	0.99
16	H	[a]	0.51
17	Me	H	>10
18	F	H	>10
19	Cl	H	12
20	Br	H	2.5
21	Me	3-CF_3	2.7
22	Me	3-Me	>10
23	F	4-OMe	1.6
24	F	3,5-$(OMe)_2$	0.79
25	F	[a]	0.44
26	Cl	[a]	0.74
27	OCF_3	[a]	1.7

[a] N-substituent is 6-fluoro-4*H*-benzo[*d*][1,3]dioxin-8-ylmethyl



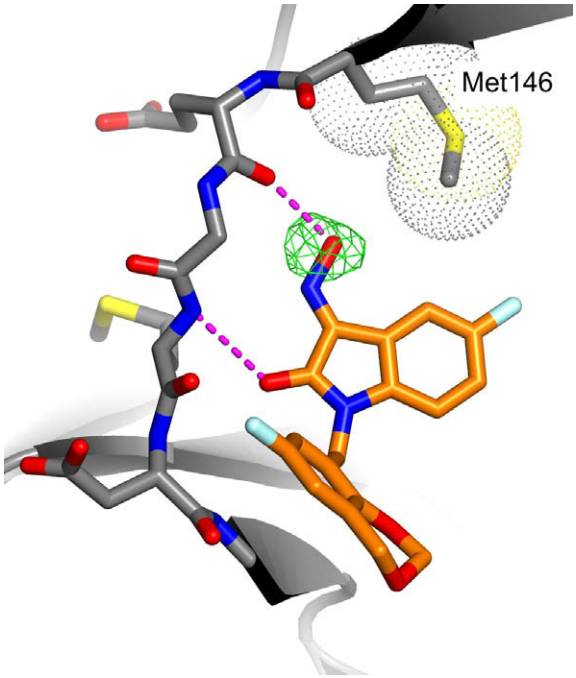
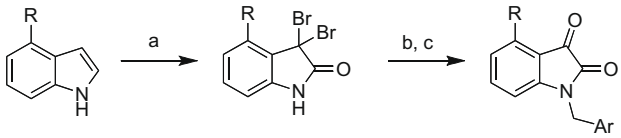


Figure 2. X-ray structure showing compound **25** bound to JNK3 active site.



Scheme 3. R = Br, CH_3, COOMe. Reagents and conditions: (a) NBS, *t*-BuOH, 2.5 h, rt; (b) MeOH, H_2O, 4 h, reflux, 44% (two steps); (c) $ArCH_2Br$, K_2CO_3, DMF, 5 h, rt, 60–70%.

Glu147. The oxime is observed in the *E*-conformation, consistent with the small molecule X-ray structure of (*E*)-*N*-(2-hydroxyethyl)-isatin oxime.[18] To determine the orientation of the oxime, a ligand lacking the oxime O was refined against the X-ray data. An electron density difference map was then calculated to show the correct placement of the oxime O (*E*- or -conformation). Vectors coming from the isatin 4- and 6-positions appeared to offer good opportunities for optimization, while those from the 5- and 7-positions present more inter- and intra-molecular steric clashes, respectively. The structure appeared to be consistent with the initial SAR of the 5-substituents. Substitution at the isatin 4-position would direct pendant groups toward a hydrophobic pocket blocked by Met-146, the gatekeeper residue that we[19] and others[13,20] have shown to be important for kinase inhibitor selectivity. A distinct difference between JNK3 and p38 is that the hydrophobic pocket in JNK3 contains a methionine (Met146) residue where the analogous residue in p38 is the less sterically demanding threonine (Thr106).[21] From the isatin 6-position, substituents would point either toward binding opportunities in the glycine-rich loop or toward solvent which would enable us to add solubilizing groups to our molecules without penalty. Given the limited commercial availability of 4 and 6-substituted isatins, we needed to prepare a selection of key intermediates.

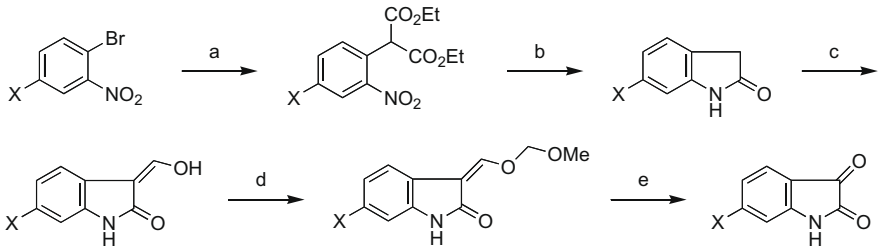
4-Substituted isatins were prepared by the method shown in Scheme 3, in which 4-substituted indoles are 3,3-dibrominated and subsequently hydrolyzed to give the desired isatin.[22]

6-Substituted isatins were prepared from the corresponding *p*-substituted *o*-nitro bromobenzenes (Scheme 4). Substitution of the bromide using diethylmalonate gave the arylmalonates, which were reduced, decarboxylated and cyclized in a single step to give the oxindole. Claisen reaction with ethyl formate gave the enol, which was O-alkylated with chloromethyl methyl ether and oxidatively cleaved to afford the 6-substituted isatins.

Further elaboration of these key intermediates into N-substituted isatin oximes was performed by the methods shown in Schemes 1 and 2.

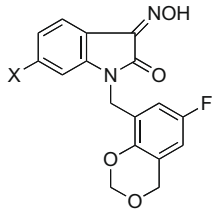
As can be seen in Table 2, results from the 6-substituted isatin series were similar to those previously observed in the 5-substituted series, in that no profound effect on binding affinity was observed. Larger substituents, designed to extend into the glycine rich loop, or out into solvent, exhibited poor binding (data not shown).

As might be expected from the structure of **25** (Fig. 2) and from previous reports on the binding of inhibitors to p38α,[19] substituents at the isatin 4-position had a more pronounced effect on binding to both JNK3 and p38α (Table 3). In comparison to the potent and selective compound **16** (X = H), the larger 4-phenyl compound **41** (Table 3) showed a slightly decreased affinity for JNK3 (1.8 μM), but, for the first time, we observed measurable affinity for p38α (9.5 μM). Figure 3 shows the X-ray crystal structure of compound **41** bound to JNK3. It can be seen that the isatin 4-phenyl substituent points towards the hydrophobic pocket, but the side chain of the gatekeeper residue Met146 is folded back, effectively closing the pocket. The isatin oxime is also seen to be in the (*Z*)-conformation. With the larger styrenyl substituent at the 4-position of compound **47** the gatekeeper residue Met 146 is forced aside (Fig. 4) to create the hydrophobic pocket that accommodates the large hydrophobic 4-styrenyl substituent. Comparison of the unsubstituted alkene **49** (JNK3 K_i = 0.14 μM) with the styrenyl compound **47** (JNK3 K_i = 0.74 μM) suggests that opening and filling the hydrophobic pocket with a rigid side chain results in slight *loss* of potency (Table 3, JNK3 data), whereas filling a corresponding hydrophobic pocket *without having to create it first* (Table 3, p38α data) results in a significant gain in potency. This loss of JNK3 activ-



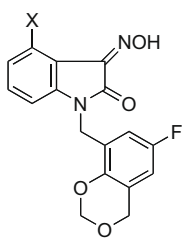
Scheme 4. Reagents and conditions (X = Me, Br, NO_2): (a) $CH_2(CO_2Et)_2$, K_2CO_3, DMF, 80 °C, 78%; (b) Fe (3 equiv), AcOH, 120 °C, 90%; (c) HCO_2Et, NaOH, EtOH, 95%; (d) CH_3OCH_2Cl, K_2CO_3, DMF, 80%; (e) $NaIO_4$, $KMnO_4$, K_2CO_3, *t*-BuOH, dioxane, 88%. Yields are indicated for X = Br.

Table 2
Data for 6-substituted isatins



Compd	6-X	JNK3 K_i (μM)[16]
16	H	0.51
28	Me	0.09
29	Br	0.1
30	Ph	0.2
31	OMe	0.24
32	CF_3	0.33
33	NH_2	0.4
34	$MeSO_2NH$	0.46
35	EtCONH	0.61
36	NO_2	0.64
37	COOMe	0.16
38	COOH	0.3
39	CONHEt	1.0

Table 3
Data for 4-substituted isatins



Compd	4-X	JNK3 K_i (μM)	p38a K_i (μM)	ERK2 K_i (μM)
16	H	0.51		
40	Br	0.185	>10	>15
41	Ph	1.8	9.5	25
42	*m*-F–Ph	5.8	7.4	23
43	*p*-F–Ph	13	3.4	>15
44	CN	2.0		
45	COOMe	30		>15
46	Me	0.7		>15
47	CH=CHPh	0.74	0.23	
48	CH_2CH_2Ph	>15	0.03	>15
49	$CH=CH_2$	0.14	8	>15
50	Et	1.4		>15

ity suggests an approximate 1 kcal net cost involved in moving the gatekeeper residue aside to create the pocket. Interestingly compound **48**, an analog of **47** with a saturated linking group, is devoid of JNK3 inhibition, yet p38α inhibition is enhanced approximately 10-fold. We attribute this to the superior ability of the rigid styryl group to shift the JNK3 Met146 side chain out of the way and the ability of the flexible phenethyl group to conform to the exact shape of the p38 hydrophobic pocket.

Compound **28** was identified as the most potent of this class of compounds and was subject to further evaluation. Selectivity for JNK family enzymes was excellent, with little or no activity observed against a panel of kinases (Table 4). Compound **28** may prove to be a useful tool compound for better understanding JNK pathway biology.

In conclusion, we have identified a novel series of JNK-selective inhibitors that exhibit a very high level of overall kinase selectivity for JNK over closely related p38 MAP kinases, as well as other kinases. A clear understanding of the mobility of the JNK3 gatekeeper Met146 residue and how this affects selectivity for this class of inhibitor was developed through the application of structure-based design. These results complement those reported by Swahn et al.[10b] for compound **51** (Fig. 5), which also exhibited an induced fit binding mode similar to that seen for compound **47**, yet showed a higher selectivity ratio for JNK3 to p38α. Compound **28** was identified as a potent selective, JNK-specific MAP kinase inhibitor that will help explore JNK biology.

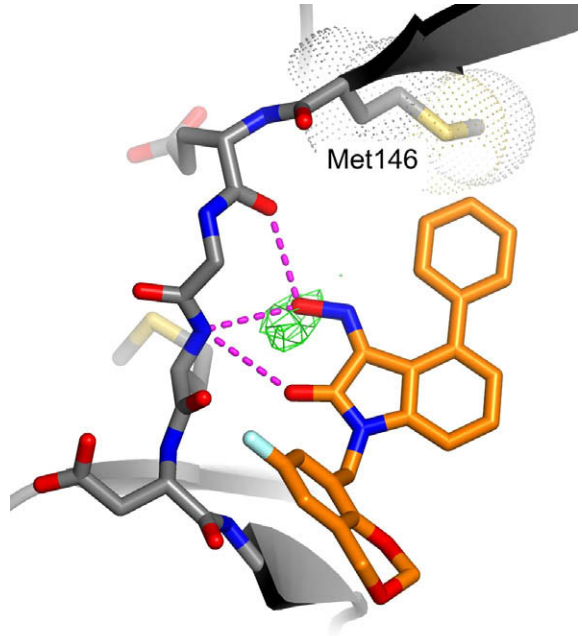


Figure 3. X-ray structure showing compound **41** bound to JNK3 active site.

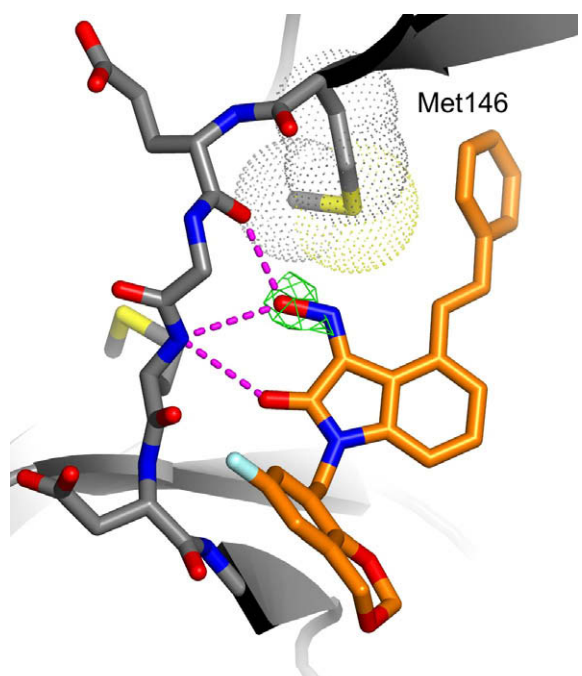


Figure 4. X-ray structure showing compound **47** bound to JNK3 active site.

Table 4
Kinase selectivity data for compound **28**

Kinase	K_i (μM)	Kinase	K_i (μM)
JNK3	0.09	JAK3	>30
JNK2	0.07	Lck	>30
JNK1	0.45	p38a	>20
AKT3	>30	PDK1	>30
CDK2	>30	ROCK1	>30
ERK2	>50	Src	>30
Flt3	>4	Syk	>30
GSK3b	>30	Tie2	>10

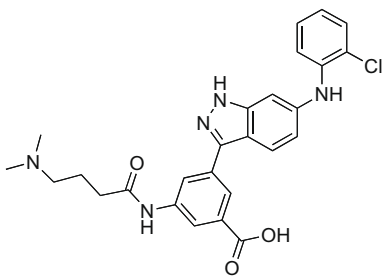


Figure 5. Compound **51**.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.043.

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