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Design and synthesis of 1-aryl-5-anilinoindazoles as c-Jun N-terminal kinase inhibitors

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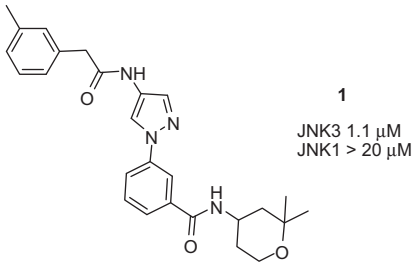
ABSTRACT

Starting from pyrazole HTS hit (**1**), a series of 1-aryl-1*H*-indazoles have been synthesized as JNK3 inhibitors with moderate selectivity against JNK1. SAR studies led to the synthesis of **5r** as double digital nanomolar JNK3 inhibitor with good in vivo exposure.

The c-Jun N-terminal kinases (JNKs) are members of mitogen-activated protein kinase (MAPK) family which are activated in response to dual phosphorylation on threonine and tyrosine when exposed to extracellular stimuli such as stress and cytokines.[1] Activation of the JNK pathway leads to the phosphorylations of a number of transcription factors involved in cell proliferation and apoptosis such as c-Jun,[2] ATF-2,[3] nuclear factor of activated T cells (NFAT),[4] and tumor suppressor p53.[1a] Ten different JNK isoforms derived from three distinct genes (jnk1, jnk2, and jnk3) have been identified. JNK1 and JNK2 are ubiquitously expressed in most tissues, while JNK3 is primarily expressed in the brain and, to a lesser extent, in the heart and testis.[5,6] This variably localized expression, together with the activation by different biochemical pathways, indicates that different JNK isoforms have distinct biological functions. For example, elevated JNK1 activity is shown to play a crucial role in the biochemical pathway responsible for obesity-induced insulin resistance in vivo,[7] whereas JNK2 and JNK3 is activated in the MPTP-induced Parkinson's disease mouse model.[8] Thus, developing JNK isoform-specific inhibitors as therapeutics has gained considerable interest over the past few years despite of the challenge that the three JNK isoforms share more than 90% sequence identity.[9,10]

As part of our effort to investigate potential therapeutical treatments for Parkinson's disease, a high throughput screening campaign was conducted to indentify JNK3 specific inhibitors. Among those, compound **1** was identified as a novel JNK3 selective inhibitor with IC_{50} values of 1.1 μM and ⩾20 μM against JNK3 and JNK1, respectively.



1

JNK3 1.1 μM
JNK1 > 20 μM

Given the floppy nature of the side chains, we sought to restrict free rotation in an effort to introduce some conformational rigidity into the molecule, and hopefully improve potency. There were two potential cyclization manifolds that would lead to conformationally restricted analogs of **1** as outlined in Figure 1. Route A removed free rotation about the phenacetyl group and led to benzimidazole **2**. Route B restricted rotation about the pyrazole linked amide to give 5-substituted indazole **3**.

Though only two compounds were synthesized, the results were undeniable (Table 1). Benzimidazole **2** showed no inhibitory activity against JNK3, while indazole **3** was a very potent JNK3 inhibitor with 10-fold selectivity against JNK1. Apparently, the 2-aminobenzimidazole in **2** failed to mimic the *m*-tolyl acetamide in **1**, whereas 5-anilinoindazole **3** succeeded perhaps indicating a conformational preference for the phenyl side chain only available to **1** and **3**.

Encouraged by the promising indazole result, our initial SAR on this scaffold was focused on modifying R^1 and R^2 as seen in compound **5** maintaining the central part of molecule as a balanced compromise between quick parallel synthesis and ability to confer JNK3 activity (Scheme 1). Starting from commercially available

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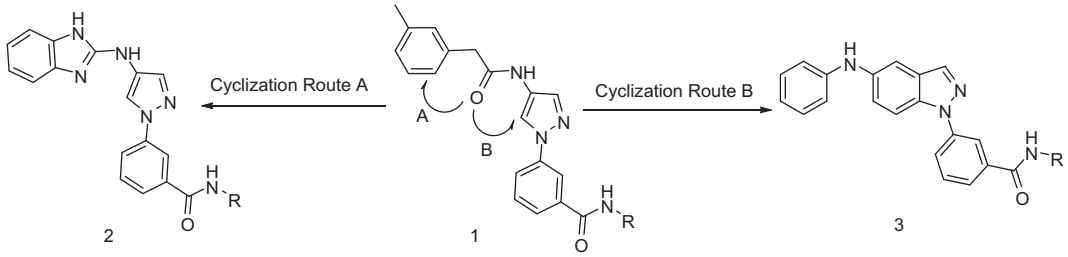
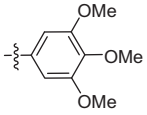
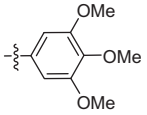


Figure 1. Potential cyclization routes from lead pyrazole **1**.

Table 1
Indazole and benzimidazole JNK inhibitors

Compd	R	JNK3[11] IC_{50}[a] (μM)	JNK1[11] IC_{50} (μM)
2		>20	NT[b]
3		0.024	0.203

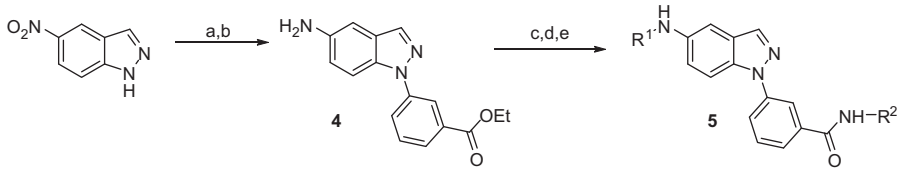
[a] The IC_{50} values are averages of two or more experiments. All standard errors ⩽12%.
[b] NT = not tested.

5-nitroindazole, Ullman coupling with ethyl 3-bromobenzoate followed by hydrogenation provided ethyl 3-(5-amino-1*H*-indazol-1-yl)benzoate (**4**) which was subjected to Buchwald amination with aryl halides (R^1). The resulting ethyl esters were hydrolyzed and coupled with various amines to furnish **5**.

Introduction of a small *ortho*-substituent like fluorine (**5a**) and chlorine (**5b**) enhanced the inhibitory activity against JNK3 compared to parent analog **3** whereas *meta*-substitution (**5d**) resulted in a small drop in potency (Table 2).[12] While the trimethoxyphenyl group in **3** was very potency enhancing, solubility and metabolic stability precluded its use in future analogs.[13] Fortunately, this part of the molecule was quite tolerant to modifications and a range of side chains were acceptable without sacrificing too much potency. Unfortunately, metabolic stability was still quite poor.[13] Compounds containing both aromatic and alkyl secondary amides exhibited good JNK3 inhibition. The morpholinopropyl analog (**5k**) was equipotent to lead indazole **3** and 3-fold more potent against JNK3 than its one carbon shorter homolog (**5g**). Actually, no side chain was needed at all as the analog containing a simple primary amide (**5p**) was quite potent against JNK3 as well. Tertiary amides and esters, on the other hand, led to 1000-fold loss of JNK3 inhibition (data not shown). This may imply the importance of the free NH group in the amide to act as a hydrogen bond donor. As seen in Table 2, this series of compounds was also somewhat selective (5- to 25-fold) for JNK3 versus JNK1 much like the pyrazole-based lead structure **1**.

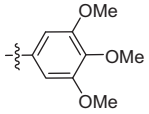
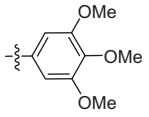
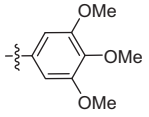
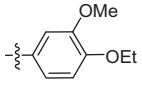
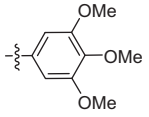
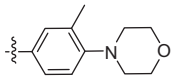
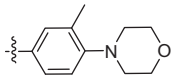
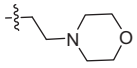
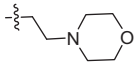
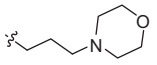
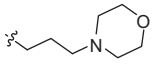
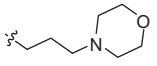
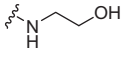
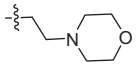
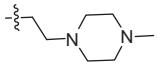
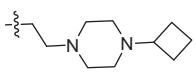
The next round of modifications involved 1-pyridyl-1*H*-indazole and 1*H*-pyrazolo[3,4]pyridine-1-yl substitutions (Table 3). Compounds **6a–f** were synthesized following chemistry detailed in Scheme 1 using the appropriate precursors. The pyrazolopyridines were made as described in the literature or purchased.[14]

The 2′- and 4′-pyridyl replacements (**6a**, **6b)** resulted in a 10-fold drop in JNK3 activity as compared to **5g** and **5h**. In these examples, an intramolecular hydrogen bond between the pyridine ring nitrogen atom and the amide side chain NH is possible to form a 5-membered ring chelate. This might disrupt hydrogen bonding between the inhibitor and the enzyme, hence, the loss in potency. Perhaps it is no coincidence that this same disruption in binding in both molecules (**6a**, **6b**) causes the same fold drop in JNK3 inhibition. With the 5′- and 6′-pyridyl analogs (**6c**, **6d**), there was only a slight loss in JNK3 inhibition (twofold) compared to phenyl derivative **5g**. Incorporation of nitrogen into the indazole ring itself led to a 2- to 3-fold drop in JNK3 inhibition (**6e–f**). All analogs tested still exhibited the 10- to 20-fold selectivity for JNK3 versus JNK1. Substitution on the *N*-phenyl pyrazole ring was conducted (2′–6′ positions, data not shown), but this did not result in any improvement in potency.[10e]



Scheme 1. Reagents and conditions: (a) ethyl 3-bromobenzoate, CuI, (1R,2R)-*N1,N2*-dimethylcyclohexane-1,2-diamine, Cs_2CO_3, Dioxane; (b) Pd/C (10%), H_2, MeOH and EtOAc; (c) R^1Br or R^1I, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, DME; (d) LiOH (1N), THF and (e) R^2NH_2, HATU, triethylamine, DMF.

Table 2
Inhibition of JNK3/1 by 1*H*-indazole benzamides

Compd	R^1	R^2	JNK3[11] IC_{50}[a] (μM)	JNK1[11] IC_{50} (μM)
3	Phenyl		0.024	0.20
5a	2-Fluorophenyl		0.009	0.073
5b	2-Chlorophenyl		0.012	0.093
5c	2-Fluorophenyl		0.046	0.43
5d	2-Methylphenyl		0.059	NT[b]
5e	2-Chlorophenyl		0.032	0.16
5f	2-Fluorophenyl		0.038	0.17
5g	2-Chlorophenyl		0.054	0.75
5h	2,6-Dichlorophenyl		0.077	1.38
5i	2-Methoxyphenyl		0.043	NT[b]
5j	2-Fluorophenyl		0.022	NT[b]
5k	2-Chlorophenyl		0.015	0.41
5l	2-Fluorophenyl		0.12	0.47
5m	2-Methylphenyl		0.25	NT[b]
5n	2-Chlorophenyl		0.035	0.80
5o	2-Pyridinyl		1.4	7.1
5p	2-Fluorophenyl	H	0.063	0.73
5q	2,6-Dichlorophenyl		0.050	1.2
5r	2-Cl,3-Me-phenyl		0.04	0.97

[a] The IC_{50} values are averages of two or more experiments. All standard errors ⩽15%.
[b] NT = not tested.

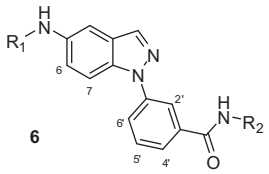


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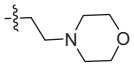
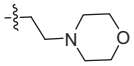
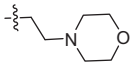
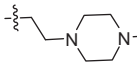
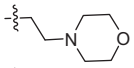
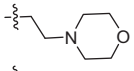
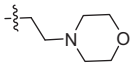
Table 3
1-Pyridyl-1*H*-indazole JNK inhibitors

Compd	Atom	R^1	R^2	JNK3[11] IC_{50}[a] (μM)	JNK1[11] IC_{50} (μM)
5g	—	2-Chlorophenyl		0.054	0.75
6a	2′-N	2-Chlorophenyl		0.64	7.01
6b	4′-N	2,6-Dichlorophenyl		0.64	NT[b]
6c	5′-N	2,6-Dichlorophenyl		0.11	NT[b]
6d	6′-N	2-Chlorophenyl		0.12	2.3
6e	6-N	2-Chlorophenyl		0.08	1.3
6f	7-N	Phenyl		0.17	1.2

[a] The IC_{50} values are averages of two or more experiments. All standard errors ⩽ 15%.
[b] NT = not tested.

While most analogs carry the amide side chain at the *meta*-position of the N1-linked phenyl ring, SAR indicates that substitution at the *para*-position is also viable (Table 4, **7**). While this minor structural modification may not have dramatic effects on JNK activity, it may play a role in brain penetration and or DMPK properties.

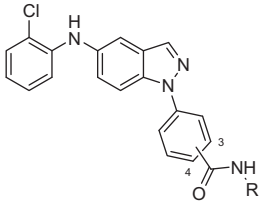
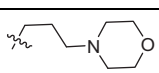
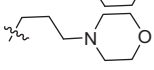


Table 4
Indazole *ortho*-, *meta*- and *para*-amide isomers

Compd	R	JNK3[11] IC_{50}[a] (μM)	JNK1[11] IC_{50} (μM)
5k		0.015	0.41
7		0.033	0.92

[a] The IC_{50} values are averages of two or more experiments. All standard errors ⩽15%.

In testing compounds for JNK3 and JNK1 inhibition, we routinely counter-screened against the closely related MAP kinase p38. Significant inhibition against p38 was observed in most compounds in this series (Table 5). Interestingly, 6-pyridyl analog **6d** showed 10-fold JNK3/p38 selectivity whereas most compounds were actually more potent against p38 than JNK3. Previously, researchers at AstraZeneca reported a related series of 6-anilinoindazole JNK3 inhibitors that also inhibit p38.[10e] Attempts to understand and improve the JNK3/p38 selectivity in this series are still ongoing.

Table 5
JNK3, JNK1 and p38 inhibition of selected compounds

Compd	JNK3[11] IC_{50}[a] (μM)	JNK1[11] IC_{50} (μM)	p38[11] IC_{50} (μM)
5a	0.009	0.073	0.03
5g	0.054	0.75	0.04
5f	0.038	0.17	0.018
6b	0.64	NT[b]	0.48
6d	0.12	2.3	1.3
6f	0.17	1.2	0.14
7	0.033	0.92	0.17

[a] The IC_{50} values are averages of two or more experiments. All standard errors ⩽25%.
[b] NT = not tested.

Table 6
In vivo profile of selected JNK3 inhibitors[16]

Compd	Clp[a] (mL/min/kg)	$t_{½}$ (min)	AUC (μM h)	*F* (%)	Plasma (μM)[b]	Brain (μM)
5a	57	1.5	0.05	5	0.79	0.05
5g	NT	NT	NT	NT	0.56	0.55
5k	123	0.5	0.01	6	0.28	0.57
5l	79	0.3	0.5	7	0.35	0.03
5q	34	2	0.38	20	2.12	0.81
5r	25	3.3	0.72	27	1.2	3.9
6d	54	0.5	0.01	5	0.53	1.4
7	NT	NT	NT	NT	0.24	0.69

[a] 1 mg/kg IV, 2 mg/kg PO.
[b] Compounds dosed 10 mpk IP; Plasma and brain drug levels measured at t = 2 h; NT = not tested.

Treating CNS disorders requires blood brain barrier (BBB) penetration.[15] Early assessment in this series with one of the more potent analogs (**5a**) was disappointing (Table 6). The other *N*-aryl amide, **5l**, also had poor CNS penetration. Fortunately, compounds containing aliphatic amide side chains (**5g**, **5k**, **5q**, **5r**, **6d**, **7**) all exhibited very good brain exposure typically with more drug in brain than in plasma at t = 2 h.[16] In terms of rat pharmacokinetics, most analogs examined had poor exposure, characterized by high clearance rates, short half-lives, and poor oral absorption. However, the two piperazine containing analogs (**5q–r**) showed markedly improved profiles. Clearance rates seem high, but this is due to higher than average volume of distributions (5–6L).[17]

Select compounds were tested in vitro for their ability to inhibit the phosphorylation of JNK3 in cells.[18] Compounds showed a considerable drop in potency (**5g** IC_{50} = 4.1 μM; **6d** IC_{50} = 5.3 μM; **5n**, **7** IC_{50}'s ⩾ 20 μM). It's not clear why there is such a large difference in biochemical versus cell-based potency as these compounds should surely penetrate the cell, however, drops in potency of this type are not uncommon in the literature.[19] This loss of potency has not been observed in all series studied, and efforts to understand this observation are on-going.

In summary, a novel series of 1-aryl-5-anilinoindazole JNK3 inhibitors were developed from the high throughput screening lead **1**. Lead compound optimization produced potent JNK3 inhibitors that showed up to 25-fold selectivity for JNK1, although 10-fold selectivity was more common. Incorporation of aliphatic amide side chains instead of aromatic amides resulted in compounds with improved PK and CNS penetration. Further optimization is required, however, to increase JNK3-p38 selectivity.

Synthesis and characterization of these compounds is in progress and will be reported in due course.

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11. Biochemical IC_{50}'s for JNK3, JNK1 and p38 were determined using HTRF. Briefly, final assay concentrations of JNK3, JNK1, biotinylated-ATF2 and ATP were 0.3, 0.1, 0.4 and 1 μM, respectively. Final assay concentrations of p38, biotinylated-ATF2 and ATP were 1, 0.4 and 11.5 μM, respectively. In both assays, the phosphor-Thr71-ATF-2 product was detected by a Europium-cryptate labeled anti-phospho-Thr71-ATF-2 antibody. Streptavidin-allophycocyanin-XL was used as the acceptor. A 10-point dose-response curve for each compound was generated in duplicate and data was fit to a four parameter logistic.
12. In a structurally related series (not yet disclosed), all 4-substituted analogs showed a drop in JNK3 inhibition. Consequently, no *para*-substituted analogs were made in this indazole series.
13. (a) The $t_{1/2}$ of **3** in rat and human liver microsomes was 5.8 and 12.8 min, respectively; (b) The $t_{1/2}$ of **5k** and **5l** in rat and human liver microsomes was: 5.6, 6.0, 8.6 min and 8.9 min, respectively.
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16. Rat pharmacokinetics and mouse brain penetration: pharmacokinetics of test compounds was assessed in Sprague–Dawley rats ($n = 3$). Compounds were dosed intravenously at 1 mg/kg and orally by gavage at 2 mg/kg. Blood was taken at eight time points (5, 15, 30 min, 1, 2, 4, 6, and 8 h) and collected into EDTA containing tubes and plasma was generated using standard centrifugation techniques. Plasma proteins were precipitated with acetonitrile and drug concentrations were determined by LC–MS/MS. Data was fit by WinNonLin using a noncompartmental model and basic pharmacokinetic parameters including oral bioavailability (F%), exposure (AUC), half-life ($t_{1/2}$), clearance (Clp), and volume of distribution (V_d) were calculated. CNS exposure was evaluated in C57Bl6 mice ($n = 3$). Compounds were dosed at 10 mg/kg intraperitoneally and after 2 h blood and brain were collected. Plasma was generated and the samples were frozen at −80 °C. The plasma and brain were mixed with acetonitrile (1:5 v/v or 1:5 w/v, respectively). The brain sample was sonicated with a probe tip sonicator to break up the tissue, and samples were analyzed for drug levels by LCMS/MS. Plasma drug levels were determined against standards made in plasma and brain levels against standards made in blank brain matrix. All procedures were approved by the Scripps Florida IACUC.
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