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Synthesis and SAR of novel quinazolines as potent and brain-penetrant c-jun N-terminal kinase (JNK) Inhibitors

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ABSTRACT

Quinazoline **3** was discovered as a novel c-jun N-terminal kinase (JNK) inhibitor with good brain penetration and pharmacokinetic (PK) properties. A number of analogs which were potent both in the biochemical and cellular assays were discovered. Quinazoline **13a** was found to be a potent JNK3 inhibitor (IC_{50} = 40 nM), with >500-fold selectivity over p38, and had good PK and brain penetration properties. With these properties, **13a** is considered a potential candidate for in vivo evaluation.

c-Jun-N-terminal Kinase (JNK) was discovered in the early-mid 1990s[1–4] and is a member of the mitogen activated protein kinase (MAP) family. In the nearly two decades of work on JNK, the enzyme has been implicated in numerous diseases ranging from cardiovascular disease,[5] to metabolic disorders[6] and neurodegeneration.[7–11] There are three isoforms of JNK. JNK1 and JNK2 are ubiquitously expressed, whereas JNK3 is expressed primarily in the brain with lower levels of expression in the heart and testis.[12]

Because of the numerous potential clinical applications for JNK, many small molecule inhibitor programs have developed over the past five years. Compound classes that have shown nice JNK selectivity include: aminopyrazoles,[13] aminopyridines,[14,15] pyridine carboxamides,[15,16] benzothien-2-yl-amides and benzothiazol-2-yl acetonitriles,[17,18] quinoline derivatives,[19] and aminopyrimidines.[20–22] For a recent review of all these classes see LoGrasso and Kamenecka.[23] Most of these classes of compounds did not demonstrate good brain penetration, although Kamenecka et al. recently reported aminopyrimidines showing excellent brain penetration properties.[22]

In the current work we present a series of novel quinazolines which were potent JNK inhibitors with >2200-fold selectivity over p38 (compound **14d**). Moreover, a systematic SAR approach utilizing biochemical and cell-based assays, along with mouse and rat pharmacokinetics enabled us to develop compounds (e.g., **13a**) which maintained their potency and selectivity (>500-fold over p38), while also incorporating good brain penetration (brain/plasma ratio of 0.8:1) in mouse, and excellent pharmacokinetics in rat. With these properties, **13a** is an attractive candidate for in vivo evaluation in CNS efficacy models.

The JNK inhibitors **1a,b** and **2** (Fig. 1) were described in the patent and primary literature,[22,24] with JNK3 IC_{50} = 90 nM for **1b** and IC_{50} = 180 nM for **2**, respectively.[22,25] The isoquinoline **1b** was only moderately potent in cells however (inhibition of c-jun phosphorylation = 1.0 μM). Compounds **1b** and **2** were found to have good brain penetration and PK properties.[22,24] As a strategy to design a novel structural class with improved JNK3 potency and similar or improved PK and brain penetration properties, we decided to combine the amino isoquinoline of compound **1** and the amino pyrimidine scaffolds (compound **2**). With this in mind we designed quinazoline **3** (Fig. 1). We found that quinazoline **3** was a potent JNK inhibitor with good brain penetration (Table 1). To establish an SAR on the quinazoline ring, we first modified the 7-position (Table 1). The synthesis is outlined in Scheme 1. For the syntheses of the 2-chloro quinazolines from the corresponding fluoro aldehydes we followed the procedure described by Patel et al.[26] A series of pyrazole, isoxazole, morpholino, and pyridyl substitutions were assessed at the 7-position on the quinazoline ring (compounds **3–8f**, Table 1). Pyrazole substitutions (compounds **3, 8a, 8g, 8h**) had the lowest JNK3 IC_{50} values, suggesting preference for pyrazole at the 7-position (Table 1). Despite the approximate three-fold improvement in cell-based potency of **8a** over **3**, the higher polar surface area of **8a** caused a significant decrease in brain penetration (Table 1). The available space for modifications in this position appeared to be very limited and we concluded that the *N*-methyl-pyrazole moiety was optimal in terms of overall

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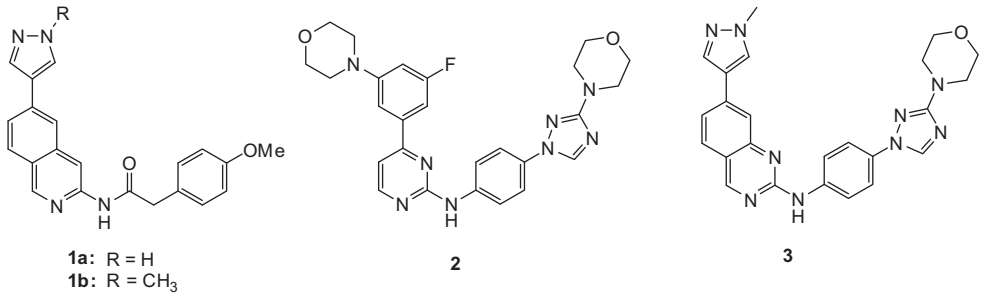
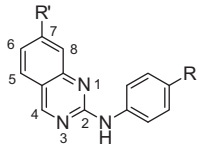
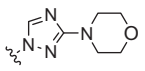
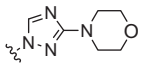
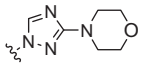
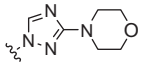
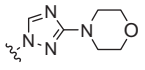
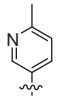
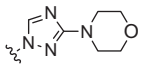
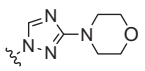
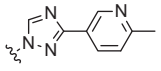
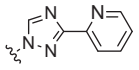


Figure 1. JNK3 inhibitors.

Table 1
Biochemical and cell-based IC_{50} values, and mouse plasma and brain levels for a series of 2,7-substituted quinazolines[a]

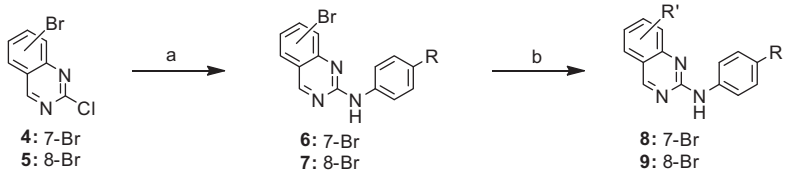


Compound	R′	R	JNK3 IC_{50} (μm)	JNK1 IC_{50} (μm)	c-Jun IC_{50} (μm)	Mouse[b] (μm)	
						Plasma	Brain
3			0.09	0.05	0.31	17.8	7.17
8a			0.05	0.04	0.12	42.9	1.31
8b			0.7	nt	nt	nt	nt
8c			4.4	nt	nt	nt	nt
8d			0.21	nt	nt	26.7	8.1
8e			0.73	nt	nt	nt	nt
8f			>20	nt	nt	nt	nt
8g			0.05	nt	0.04	nt	nt
8h			0.06	0.1	0.29	13.1	1.37

nt = not tested.

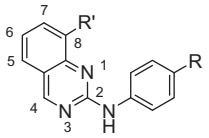
[a] JNK3 biochemical IC_{50} values are the averages of four or more experiments, and the JNK1 and cell-based IC_{50} values are the averages of two or more experiments. All standard deviations are ⩽44% for the biochemical and ⩽81% for the cell-based assays.

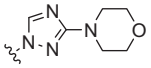
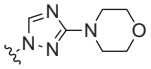
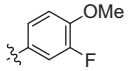
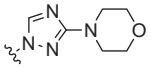
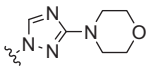
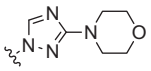
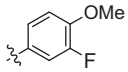
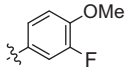
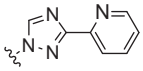
[b] 10 mg/kg ip 2 h.



Scheme 1. Reagents and conditions: (a) HCl in EtOH, *n*-BuOH, 120 °C, 2–24 h; (b) Boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/water, 120 °C, 30', μW.

Table 2
Biochemical and cell-based IC_{50} values, and mouse plasma and brain levels for a series of 2,8-substituted quinazolines[a]



Compound	R′	R	JNK3 IC_{50} (μm)	JNK1 IC_{50} (μm)	c-Jun IC_{50} (μm)	Mouse[b] (μm)	
						Plasma	Brain
9a			0.03	0.02	0.02	2.7	0.15
9b			0.02	0.01	0.33	2.2	0.23
9c			0.07	0.09	0.25	6.1	1.5
9d			0.007	0.03	3.0	nt	nt
9e			0.06	0.05	1.4	5.2	12.7
9f			0.08	nt	0.5	10.3	7.6
9g			0.02	0.01	0.2	5.0	4.5
9h			0.09	nt	5.1	nt	nt

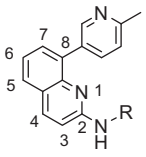
nt = not tested.
[a] JNK3 biochemical IC_{50} values are the averages of four or more experiments, and the JNK1 and cell-based IC_{50} values are the averages of two or more experiments. All standard deviations are ⩽44% for the biochemical and ⩽81% for the cell-based assays.
[b] 10 mg/kg ip 2 h.

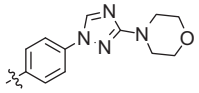
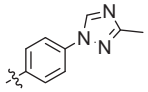
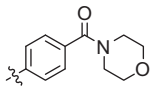
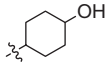
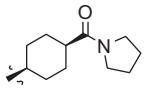
properties. Replacement of the 3-morpholino on the 1,2,4-triazole (**8a**) with 3-*p*-methyl-pyridyl on the 1,2,4-triazole (**8g**) decreased the cell-based IC_{50} by three-fold to 40 nM. However, this substitution caused brain penetration to be quite poor (**8h**) (Table 1), especially when compared to compound **3**. Attempts to improve the potency and maintain the good pharmacological profile by replacing the morpholino-triazolo aniline moiety were unsuccessful (data not shown).

We next investigated the 8-position on the quinazoline ring (Table 2). We found a number of different substitutions that improved the biochemical potency. Compound **9a** showed good cellular potency but unfortunately the brain penetration was poor (Table 2). Similarly, the thiazole replacement at position 8 improved the JNK3 potency by four-fold (compare **9d** to **9a**), but the cell-based IC_{50} for **9d** was 150-fold less potent than **9a** suggesting decreased cell penetration for **9d**. By replacing the methyl pyridine group with phenyl groups and thus reducing the polar surface area we indeed did achieve much improved brain penetration (compounds **9e**/**f**), but unfortunately these compounds were only modestly active in cells (Table 2). Compound **9g** did have both good brain penetration and potency (Table 2); however the PK properties were poor (data not shown).

In an effort to improve the brain penetration and also PK properties of **9a** we further explored the effect of substitutions in the 2-position (Table 3). We hypothesized that by introducing groups in the 2-position that would reduce the polar surface area and molecular weight we would improve the brain penetration. Compound **10b** indeed showed much improved brain to plasma ratio compared to **9a**; however the plasma concentration was low presumably due to high clearance. Furthermore, the good biochemical potency did not translate into good cellular potency (Table 3). Despite extensive synthetic efforts we were unable to achieve the good PK and brain penetration properties we had with quinazoline **3** by modifying the 2-, 7- and 8- positions. Generally, the 8-substituted quinazolines led to more potent compounds but poorer PK properties. A contributing factor may be the significantly shorter half lives in microsomes as compared to the 7-substituted analogs. It became apparent that compounds with the *N*-methyl pyrazole group in the 7-position led to compounds with good pharmacological properties and substitutions in the 8-position improved the potency. We should also note that substitutions in the 5- and 6-positions were generally not well tolerated (data not shown). We therefore focused on the synthesis of analogs bearing the *N*-methyl pyrazole group in the 7-position and different substitutions in the 8-position (Table 4). All compounds shown in Table 4 were synthesized as outlined in Scheme 2, except for compounds **14b** and **14f**, which were made in five steps starting from formylation of 3-fluoro-2-(trifluoromethyl)bromobenzene and 2-bromo-6-fluoroanisole, respectively. Compound **14d** showed the best JNK3 potency with IC_{50} = 9 nM and cell-based IC_{50} = 40 nM, and had great selectivity over p38 (IC_{50} >20 μM), but unfortunately had very poor brain penetration (Table 4). The great selectivity of compound **14d** for JNK3 over p38 can potentially be attributed to a better fit in the smaller active site for JNK3 and/or a planar structure to the molecule. Compound **14f** was found to be very potent (IC_{50} = 4 nM vs JNK3) with good brain penetration; however its oral bio-availability was low (%*F* = 8) and therefore not a good candidate for further advancement. The same was true for

Table 3
Biochemical and cell-based IC_{50} values, and mouse plasma and brain concentrations for 2-position substitutions designed to reduce polar surface area and molecular weight[a]

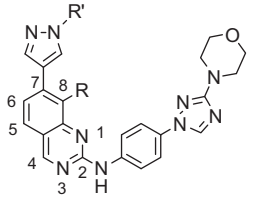


Compound	R	JNK3 IC_{50} (μm)	c-Jun IC_{50} (μm)	Polar surface area[b]	Mouse[c] (μm) Plasma	Brain
9a		0.03	0.02	90	2.72	0.15
10a		0.03	nt	77	2.31	0.23
10b		0.06	0.63	68	0.78	1.06
10c		0.17	nt	49	nt	nt
10d		0.09	nt	79	0	0
10e		0.05	0.93	69	nt	nt
10f		0.03	3.0	69	nt	nt

[a] JNK3 biochemical IC_{50} values are the averages of four or more experiments, and the JNK1 and cell-based IC_{50} values are the averages of two or more experiments. All standard deviations are ⩽44% for the biochemical and ⩽81% for the cell-based assays.
[b] Polar surface area calculated by ChemBioDraw Ultra 11.0.
[c] 10 mg/kg ip 2 h.

many of the other compounds with good brain penetration in the **14** series. We decided to introduce a chloro substitution at position 8 on the quinazoline ring and identified compound **13a**, which had

Table 4
Biochemical and cell-based IC_{50} values, and mouse plasma and brain levels for a series of 2,7,8-substituted quinazolines[a]

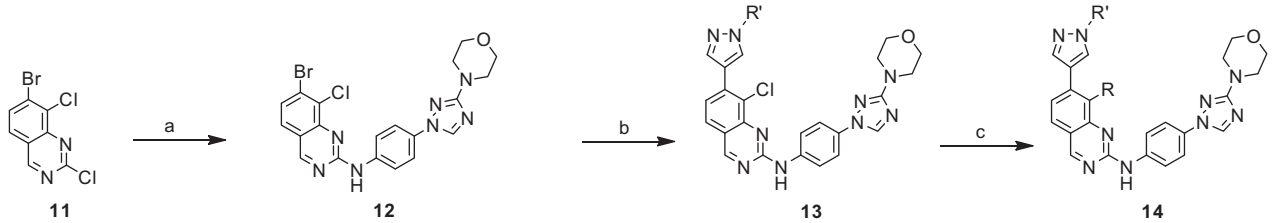


Compound	R	R′	JNK3 IC_{50} (μm)	c-Jun IC_{50} (μm)	Mouse[b] (μm) Plasma	Brain
13a	Cl	CH_3	0.04	0.10	10.4	8.3
13b	Cl		0.063	0.62	4.3	7.2
14a	CH_3	CH_3	0.01	0.16	7.4	7.6
14b	CF_3	CH_3	0.19	nt	8.3	5.6
14c		CH_3	0.02	0.13	10.6	5.0
14d		CH_3	0.009	0.04	6.3	0.22
14e		CH_3	0.01	0.04	6.4	1.1
14f	OMe	CH_3	0.004	0.05	9.4	4.9

nt = not tested.
[a] The JNK3 biochemical IC_{50} values are the averages of four or more experiments, the JNK1 and cell-based IC_{50} values are the averages of two or more experiments. All standard deviations are ⩽44% for the biochemical and ⩽81% for the cell-based assays.
[b] 10 mg/kg ip 2 h.

excellent brain penetration and good PK properties as well (Tables 4 and 5). Moreover, the compound had >500-fold selectivity over p38. Compound **13b** had a superior brain/plasma ratio of 1.7:1 compared to **13a**, but suffered from a six-fold less potent cell-based activity compared to **13a** (Table 4). The PK properties of compounds **3** and **13a** are summarized in Table 5. Compound **13a** is currently under further investigation in animal models.

In summary, we discovered a series of novel quinazoline compounds as JNK inhibitors with good potency in biochemical and cellular assays. Compound **13** improved upon compound **3** by three-fold in cell potency and had 80% brain penetration compared to 40% for compound **3**. In contrast, compound **3** had a greater



Scheme 2. Reagents and conditions: (a) HCl in EtOH, *n*-BuOH, 120 °C, 2–24 h; (b) boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/water, 120 °C, 30', μW; (c) boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane/water, 100–140 °C, 12–16 h, μW.

Table 5
PK properties of the two lead compounds

Compound	Clp (mL/min/kg)	Rat pk[a] $t_{1/2}$ (h)	V_d (L)	Oral AUC (μM∗h)	Oral C_{max} (μM)	%F	Mouse[b] (μm) Plasma	Brain
3	3	3	0.8	6.3	0.7	28	17.8	7.2
13a	6	3	1.4	2.3	0.3	19	10.4	8.3

[a] 1 mg/kg IV, 2 mg/kg po.
[b] 10 mg/kg ip 2 h.

systemic exposure and oral bioavailability suggesting that compound **3** may be preferred for non-central nervous system (CNS) indications and compound **13** may be preferred for CNS indications.

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