Preparation of 6-Substituted Quinoxaline JSP-1 Inhibitors by Microwave Accelerated Nucleophilic Substitution

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Abstract: A small library of 6-aminoquinoxalines has been prepared by nucleophilic substitution of 6-fluoroquinoxaline with amines and nitrogen-containing heterocycles under computer-controlled microwave irradiation. Some compounds were found to be potent inhibitors of JNK Stimulatory Phosphatase-1 (JSP-1) in an in vitro biological assay.

Keywords: Microwave irradiation; nucleophilic substitution; 6-substituted quinoxalines; JSP-1.

Introduction

Quinoxalines are an important class of nitrogen-containing heterocycles with a variety of biological activities, specifically as AMPA/GlyN receptor antagonists [1-3], angiotensin II receptor antagonists [4-5], anticancer agents [6-9], antiinfection agents [10-11] and immunomodulating agents [12-13].

Through a high-throughput screening of our sample collection, an aminoquinoxaline derivative I (Figure 1) was found to show inhibitory activity towards JNK Stimulatory Phosphatase-1 (JSP-1) in an in vitro biological assay [14]. JSP-1, also known as VHX/MKPX [15] or JKAP [16], is a special
member of the so-called Dual-Specificity Protein Phosphatases (DSPs) family [17], a class of protein tyrosine phosphatases (PTPs), which are a family of intracellular enzymes that dephosphorylate proteins with phosphate on serine, threonine and/or tyrosine, and play important regulative roles in cellular signal transduction. Unlike other DSPs, which directly inactivate mitogen-activated protein kinases (MAPK), JSP-1 is a selective activator of the MAPK Jun NH2-terminal kinase (JNK). The JNK signal transduction pathway is implicated in many pathological conditions, including cancer, diabetes and neurodegenerative diseases [18-19], therefore, JSP-1 might be a novel potential therapeutic target for treating diseases associated with dysfunctional JNK signaling.

Figure 1.

According to the literature 6-aminoquinoxalines could be prepared by condensation of corresponding ortho-phenylenediamines with 1,2-dioxo compounds [20-21] or by nucleophilic substitution of haloquinoxalines [22-26]. Since appropriately substituted ortho-phenylenediamines are not always readily available, the former route is not very useful for the construction of 6-aminoquinoxaline libraries and consequently, the latter one was considered a more attractive option to synthesize the target compounds. However, the nucleophilic substitution reaction, which affords 26-88% yields of the target compounds after reactions at high temperature for 3-22 hours, usually requires an electron-withdrawing substituent such as a fluorine atom or nitro group at the ortho-position of the 6-halo substituted quinoxalines [22-25]. There is one report claiming that 2,3-dimethyl-6-chloroquinoxaline or methyl-6-fluoroquinoxaline reacted with methylamine in sealed tube for 16-26 hours to produce the expected product in 63-80% yield [26].

Recently, microwave-enhanced organic reactions have become quite attractive in organic synthesis [27-33]. The main advantages of this protocol are the potential to improve the yields and dramatically shorten the reaction times. Herein, we would like to report a microwave-assisted synthetic method to synthesize 6-aminoquinoxalines using 6-fluoroquinoxalines and amines or nitrogen-containing heterocycles in a single-mode microwave synthesizer.

Scheme 1. Nucleophilic substitution of 6-fluoroquinoxalines with amines or nitrogen-containing heterocycles.

Reagents: (a) H2, 10% Pd/C, EtOH. (b) R1 COCOR1, EtOH, reflux. (c) HNR2R3, base.
Results and Discussion

Chemistry

The synthetic route used is outlined in Scheme 1. 4-Fluoro-2-nitroaniline was reduced by catalytic hydrogenation in the presence of 10% Pd/C to afford 4-fluoro-o-phenylenediamine (1) [34]. Condensation of compound 1 with 1,2-dioxo compounds gave the 6-fluoroquinoxalines 2a-d. As a model reaction to optimize the reaction conditions for preparing compounds 3-22, the synthesis of 6-(1-pyrrolidinyl)-quinoxaline (3) was examined in some detail. First, 1 equivalent of 6-fluoroquinoxaline (2a) was refluxed with 2 equivalents of pyrrolidine in the presence of 2 equivalents of potassium carbonate in dimethyl sulfoxide (DMSO). After 30 min, the resulting complex reaction mixture was checked by HPLC, which showed 21% of unreacted starting material 2a and 16% of the expected product 3 (Table 1, entry 1). When the reflux time was prolonged to 3 hours (Table 1, entry 2), the disappearance of the starting material 2a was confirmed by HPLC, but the yield of substituted product 3 was only slightly increased to 22% and more side products were formed.

Table 1. Optimization of reaction conditions.

| Entry | 2a (equiv.) | Pyrrolidine (equiv.) | Base       | Solvent | T (°C) | t (min) | Yield of 3 (%) |
|-------|-------------|---------------------|------------|---------|--------|---------|---------------|
| 1     | 1           | 2                   | K₂CO₃      | DMSO    | 189    | 30      | 16⁵          |
| 2     | 1           | 2                   | K₂CO₃      | DMSO    | 189    | 180     | 22⁶          |
| 3     | 1           | 2                   | K₂CO₃      | DMF     | 120    | 30      | 22⁵          |
| 4     | 1           | 2                   | K₂CO₃      | NMP     | 200    | 30      | 55⁶          |
| 5     | 1           | 2                   | K₂CO₃      | DMSO    | 200    | 30      | 93⁵          |
| 6     | 1           | 2                   | DBU        | DMSO    | 200    | 30      | 30⁵          |
| 7     | 1           | 2                   | NaOH       | DMSO    | 200    | 30      | 80⁵          |
| 8     | 1           | 1                   | K₂CO₃      | DMSO    | 200    | 30      | 30⁵          |
| 9     | 1           | 2                   | K₂CO₃      | DMSO    | 180    | 30      | 71⁵          |
| 10    | 1           | 2                   | K₂CO₃      | DMSO    | 220    | 30      | 83⁵          |
| 11    | 1           | 2                   | K₂CO₃      | DMSO    | 200    | 20      | 70⁵          |

⁵ The yields were determined by HPLC and LC–MS spectroscopy of the crude reaction mixture.
⁶ The reactions were carried out with stirring under refluxing in an oil bath.
⁷ The reactions were carried out with stirring under microwave irradiation.

To improve the nucleophilic substitution reaction, microwave irradiation was then employed, and the optimum reaction conditions for this reaction were investigated. As the results presented in Table 1 show, when a mixture of 1 equivalent of 6-fluoroquinoxaline (2a), 2 equivalents of pyrrolidine and 2 equivalents of potassium carbonate in N,N-dimethylformamide (DMF) was exposed to microwave irradiation at 120 °C for 30 min (Table 1, entry 3), the yield of substituted product 3 was not improved and considerable starting material 2a was detected. When the reaction temperature was raised to 200 °C and the solvent was changed from DMF to N-methyl pyrrolidone (NMP) or DMSO, a moderate
yield improvement was observed for the reaction in NMP (Table 1, entry 4), but the reaction was complete within 30 min in DMSO to give the best yield (93%) (Table 1, entry 5). Based on these reactions DMSO was selected as the solvent for subsequent work. The effect of the base, sodium hydroxide or 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU), was also investigated, but no yield improvements were observed (Table 1, entries 6-7) and more side products were detected in both cases. When the amount of pyrrolidine used was reduced to one equivalent, the yield also decreased (Table 1, entry 8). Distinctly, the concentration of nucleophile played an important role in the outcome of the reactions. The yield was not improved regardless of whether the reaction temperature was raised or decreased. It was observed that the reaction was incomplete at temperatures lower than 200 °C (Table 1, entry 9) and more by-products were formed at higher temperature (Table 1, entry 10). Shortening the reaction time also resulted in a decrease in the yield (Table 1, entry 11).

Following the results described above, it is suggested that the optimal reaction conditions involve a mixture of one equivalent of 6-fluoroquinoxaline (2a), two equivalents of pyrrolidine and two equivalents of potassium carbonate in DMSO exposed to microwave irradiation at 200°C for 30 min and that microwave irradiation might be a pivotal driving force to accelerate the nucleophilic substitution.

To extend the utility of the reaction, the above method was employed to synthesize other 6-amino-quinoxalines, and the reactions of substituted 6-fluoroquinoxalines and various amines were investigated (Table 2). It could be shown that the reaction yields depended on the character of the nucleophilic reagents used. When nitrogen-containing aromatic heterocycles were employed as nucleophiles, excellent yields (88-97%) were obtained. When aliphatic amines were reacted with 6-fluoroquinoxalines, both nucleophilic and steric effects influenced the yields and reaction rates. No good reaction occurred with primary amines, except in the case of 4-methoxybenzylamine, but when secondary amines were used, moderate to good yields were obtained. Changing from pyrrolidine to six-membered cyclic secondary amines, a decrease in yields was noted, which may be attributed to steric hindrance. Meanwhile, an effect of the substituents on the quinoxaline ring was also observed. 2,3-Difuryl substituted 6-fluoroquinoxaline reacted with cyclic secondary amines to give excellent yields of product within 30 min following the general procedure, but the reaction times had to be prolonged to complete the reaction of 2,3-dimethyl- or 2,3-di-p-tolyl- substituted 6-fluoroquinoxaline with amines.

Table 2. Nucleophilic substitution of 6-fluoroquinoxalines with amines and nitrogen-containing aromatic heterocycles.

| Entry | Compound | R¹ | NR²R³ | Yield (%) a |
|-------|----------|----|-------|-------------|
| 1     | 3        | H  | Pyrrolidine | 93          |
| 2     | 4        | H  | Piperidine  | 85          |
| 3     | 5        | H  | 4-Methoxybenzylamine | 45          |
| 4     | 6        | H  | Imidazole    | 92b         |
| 5     | 7        | H  | Pyrazole     | 88b         |
| 6     | 8        | Methyl | Pyrrolidine  | 88c         |
| 7     | 9        | Methyl | Piperidine  | 50c         |
Table 2. Cont.

| Entry | Compound             | R² | NR³R³             | Yield (%) \(^a\) |
|-------|----------------------|----|-------------------|-----------------|
| 8     | 10                   | Methyl | Imidazole         | 88              |
| 9     | 11                   | Methyl | Pyrazole          | 88              |
| 10    | 12                   | 2-Furyl | Pyrrolidine      | 93              |
| 11    | 13                   | 2-Furyl | Piperidine       | 90              |
| 12    | 14                   | 2-Furyl | Homopiperidine   | 73              |
| 13    | 15                   | 2-Furyl | 4-Methoxybenzylamine | 80          |
| 14    | 16                   | 2-Furyl | Pyrrole          | 94              |
| 15    | 17                   | 2-Furyl | Imidazole        | 97              |
| 16    | 18                   | 2-Furyl | Pyrazole         | 97              |
| 17    | 19                   | 4-Methylphenyl | Pyrrolidine | 90\(^c\)         |
| 18    | 20                   | 4-Methylphenyl | Homopiperidine | 50\(^c\)      |
| 19    | 21                   | 4-Methylphenyl | Pyrrole        | 96              |
| 20    | 22                   | 4-Methylphenyl | Pyrazole      | 96              |

\(^a\) Isolated yields of pure compounds.

\(^b\) Microwave irradiation, 200 °C, 5 min

\(^c\) Microwave irradiation, 200 °C, 60 min

**Biological activity**

All the synthesized compounds were assayed *in vitro* for their inhibiting activities towards JSP-1, and a number of these 2,3-diaryl substituted quinoxalines exhibited potent inhibitory activity, with compound 16 displaying the most potent activity.

Table 3. JSP-1 inhibiting activities of 6-aminoquinoxalines 12-22.

| Entry | Compound             | R² | NR³R³             | JSP-1 IC\(_{50}\) (µM)\(^a\) |
|-------|----------------------|----|-------------------|-----------------|
| 1     | 12                   | 2-Furyl | Pyrrolidine      | 7.51±0.81       |
| 2     | 13                   | 2-Furyl | Piperidine       | 4.18±0.03       |
| 3     | 14                   | 2-Furyl | Homopiperidine   | 5.37±0.53       |
| 4     | 15                   | 2-Furyl | 4-Methoxybenzylamine | 6.44±1.02   |
| 5     | 16                   | 2-Furyl | Pyrrole          | 2.61±0.34       |
| 6     | 17                   | 2-Furyl | Imidazole        | 4.77±0.32       |
| 7     | 18                   | 2-Furyl | Pyrazole         | 8.03±0.99       |
| 8     | 19                   | 4-Methylphenyl | Pyrrolidine | 31.62±1.84     |
| 9     | 20                   | 4-Methylphenyl | Homopiperidine | 9.20±0.65     |
| 10    | 21                   | 4-Methylphenyl | Pyrrole        | 9.23±0.90       |
| 11    | 22                   | 4-Methylphenyl | Pyrazole      | 42.44±4.80      |
| 12    | Reference            |     | 3-((5-((4-fluorophenyl)methylene)-4-oxo-2-thioxo)-3-thiazolidinyl)-benzoic acid [35] | 15.37±0.13 |

\(^a\) Data are means of three independent experiments
Conclusions

In summary, an efficient method was developed to prepare 6-aminoquinoxalines in moderate to excellent yields within 5-60 min under microwave irradiation, whereas with standard heating this reaction could not be completed even after prolonged reaction times and a complex mixture of by-products was formed. To extend the broad applicability of this procedure, this microwave assisted nucleophilic substitution was employed to construct a small parallel library of 6-substituted quinoxalines, which exhibited JSP-1 inhibiting activities in an *in vitro* enzymatic assay.

Experimental Section

General

The reagents were purchased from Lancaster (Morecambe, England), Aldrich (St. Louis, MO, USA), Acros (Geel, Belgium) and Shanghai Chemical Reagent Company (Shanghai, China), and were used without further purification. The single-mode microwave synthesizer employed for this work was an Initiator from Biotage (Uppsala, Sweden), which is equipped with an internal probe that monitors reaction temperature and pressure, and maintains the desired temperature by computer control. Reactions were conducted in the 5 mL sealed vials. Analytical thin-layer chromatography was performed on HSGF 254 plates (150–200 µm thickness; Yantai Huiyou Company, Yantai, Shandong, China). Preparative thin-layer chromatography was carried out on HSGF 254 plates (400–500 µm thickness; Yantai Huiyou Company, Yantai, Shandong, China). Column chromatography was performed using 200-300 mesh silica gels (Qingdao Haiyang Chemical Company, Qingdao, Shandong, China). Yields were not optimized. Melting points were recorded in a capillary tube on a SGW X-4 melting point apparatus without correction. ^1^H-NMR was recorded in CDCl3 on a Varian AMX-300 (300 MHz) NMR spectrometer using tetramethylsilane as an internal standard. Chemical shifts were reported in parts per million (ppm, δ). Proton coupling patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), and broad (br). Mass spectra (MS) were measured by the electric ionization (EI) method with a Finnigan MAT-95 instrument (Finnigan, Santa Clara, CA, USA).

General procedure for the preparation of 6-fluoroquinoxalines 2a-d: Preparation of 6-fluoroquinoxaline (2a)

4-Fluoro-2-nitroaniline (2.0 g, 0.013 mol), dissolved in ethanol (80 mL), was hydrogenated in the presence of 10% Pd/C (800 mg) under 1 atm H2 at room temperature. When the hydrogen uptake was complete the catalyst was removed by filtration and a mixture of the filtrate and 40% glyoxal in water (1.5 mL, 0.013 mol) was heated at reflux for 24 hours. The solvent was evaporated under vacuum to give the crude product, which was further purified by flash column chromatography to give the desired compound 2a (1.0 g, yield 53 %) as a yellow solid. mp: 33-35 °C; ^1^H-NMR: δ 8.87 (1H, d, J=1.8 Hz), 8.85 (1H, d, J=1.8 Hz), 8.12~8.17 (1H, m), 7.74~7.78 (1H, m), 7.56~7.63 (1H, m); EIMS m/z: 148 (M^+^). The following compounds were similarly prepared:
2,3-dimethyl-6-fluoroquinoxaline (2b): A yellow solid, yield 57%; mp: 108-110°C; \(^1\)H-NMR: \(\delta\) 7.95-8.00 (1H, m), 7.59-7.63 (1H, m), 7.41-7.48 (1H, m), 2.74 (3H, s), 2.73 (3H, s); EIMS m/z: 176 (M\(^+\)).

2,3-di(2-furanyl)-6-fluoroquinoxaline (2c): A yellow solid, yield 72%; \(^1\)H-NMR: \(\delta\) 8.12-8.17 (1H, m), 7.76-7.80 (1H, m), 7.64-7.66 (2H, m), 7.51-7.57 (1H, m), 6.66-6.71 (2H, m), 6.57-6.60 (2H, m); EIMS m/z: 280 (M\(^+\)).

2,3-di(4-methylphenyl)-6-fluoroquinoxaline (2d): A yellow solid, yield 76%, mp: 114-116°C; \(^1\)H-NMR: \(\delta\) 8.12-8.17 (1H, m), 7.75-7.79 (1H, m), 7.49-7.55 (1H, m), 7.40-7.17 (4H, m), 6.90 (1H, m), 3.44-3.49 (4H, m), 2.07-2.12 (4H, m); EIMS m/z: 328 (M\(^+\)).

**General procedure for the preparation of 6-aminoquinoxalines 3-22 under microwave irradiation: Synthesis of 6-(1-pyrroldinyl)-quinoxaline (3).**

Pyrrolidine (112 \(\mu\)L, 97 mg, 1.36 mmol) was added in a 5 mL microwave vial containing 6-fluoroquinoxaline (100 mg, 0.68 mmol) and K\(_2\)CO\(_3\) (188 mg, 1.36 mmol) in DMSO (1.5 mL). The vial was sealed by a crimped cap and was placed in a Biotage microwave apparatus. The vessel was heated for 30 min at 200 °C. After completion of the reaction, the reaction mixture was cooled and the mixture was poured into ice-water (50 mL). The mixture was extracted three times with dichloromethane and the combined organic layers were washed with brine, dried over anhydrous sodium sulfate and filtered. The solvent was evaporated under vacuum, and the resulting crude product was purified by preparative thin-layer chromatography to give 125 mg of the title compound 3 as a yellow solid, yield 93%; mp: 77-79°C; \(^1\)H-NMR: \(\delta\) 8.62 (1H, d, \(J=1.8\) Hz), 8.46 (1H, d, \(J=1.8\) Hz), 7.87-7.91 (1H, m), 7.22-7.26 (1H, m), 6.90 (1H, m), 3.44-3.49 (4H, m), 2.07-2.12 (4H, m); EIMS m/z: 199 (M\(^+\)); HRMS (EI): C\(_{12}\)H\(_{13}\)N\(_3\) Calculated: 199.1110; Found 199.1108. The following compounds were similarly prepared:

6-(1-piperidinyl)-quinoxaline (4): A yellow oil, yield 85%; \(^1\)H-NMR: \(\delta\) 8.67 (1H, d, \(J=1.8\) Hz), 8.56 (1H, d, \(J=1.8\) Hz), 7.89-7.92 (1H, m), 7.52-7.56 (1H, m), 7.26 (1H, m), 3.39-3.42 (4H, m), 1.66-1.79 (6H, m); EIMS m/z: 213 (M\(^+\)); HRMS (EI): C\(_{13}\)H\(_{15}\)N\(_3\) Calculated: 213.1266; Found 213.1266.

4-(methoxy)-N-6-quinoxalinyl-benzenemethanamine (5): A yellow solid, yield 45%; mp: 118-120°C; \(^1\)H-NMR: \(\delta\) 8.63 (1H, d, \(J=1.5\) Hz), 8.51 (1H, d, \(J=1.5\) Hz), 7.83-7.85 (1H, m), 7.32-7.34 (2H, m), 7.13-7.16 (1H, m), 7.00 (1H, m), 6.89-6.91 (2H, m), 4.39 (2H, s), 3.80 (3H, s); EIMS m/z: 265 (M\(^+\)); HRMS (EI): C\(_{16}\)H\(_{15}\)N\(_3\)O Calculated: 265.1215; Found 265.1207.

6-(1H-imidazol-1-yl)-quinoxaline (6): A yellow solid, yield 92%; mp: 197-199°C; \(^1\)H-NMR: \(\delta\) 8.93 (1H, d, \(J=2.1\) Hz), 8.91 (1H, d, \(J=2.1\) Hz), 8.27 (1H, d, \(J=9.0\) Hz), 8.13 (1H, d, \(J=2.7\) Hz), 8.08 (1H, dd, \(J=2.7\) Hz, \(J=9.0\) Hz), 7.87-7.91 (1H, m), 7.49 (1H, m), 7.31 (1H, m); EIMS m/z: 196 (M\(^+\)); HRMS (EI): C\(_{11}\)H\(_8\)N\(_4\) Calculated: 196.0749; Found 196.0734.
6-(1H-pyrazol-1-yl)-quinoxaline (7): A yellow solid, yield 88%; mp: 75-78°C; $^1$H-NMR: $\delta$ 8.89 (1H, d, J=2.0 Hz), 8.85 (1H, d, J=2.0 Hz), 8.35–8.38 (1H, m), 8.30 (1H, m), 8.20–8.23 (1H, m), 8.15 (1H, m), 7.84 (1H, m), 6.58 (1H, m); EIMS m/z: 196 (M$^+$); HRMS (EI): C$_{11}$H$_8$N$_4$ Calculated: 196.0749; Found 196.0746.

2,3-dimethyl-6-(1-pyrrolidinyl)-quinoxaline (8): A yellow solid, yield 88%; mp: 168-171°C; $^1$H-NMR: $\delta$ 7.80 (1H, d, J=9.0 Hz), 7.13 (1H, dd, J=9.0 Hz, J=2.7 Hz), 6.85 (1H, d, J=2.7 Hz), 3.41–3.46 (4H, m), 2.66 (3H, s), 2.65 (3H, s), 2.05–2.10 (4H, m); EIMS m/z: 227 (M$^+$); HRMS (EI): C$_{14}$H$_{17}$N$_3$ Calculated: 227.1423; Found 227.1420.

2,3-dimethyl-6-(1-piperidinyl)-quinoxaline (9): A yellow solid, yield 50%; mp: 104-106°C; $^1$H-NMR: $\delta$ 7.80 (1H, d, J=9.1 Hz), 7.44 (1H, dd, J=9.1 Hz, J=2.6 Hz), 7.22 (1H, d, J=2.6 Hz), 3.84–3.87 (2H, m), 2.81–2.90 (2H, m), 2.67 (3H, s), 2.66 (3H, s), 1.75–1.80 (2H, m), 1.34–1.40 (4H, m); EIMS m/z: 241 (M$^+$); HRMS (EI): C$_{15}$H$_{19}$N$_3$ Calculated: 241.1579; Found 241.1584.

2,3-dimethyl-6-(1H-imidazol-1-yl)-quinoxaline (10): A yellow solid, yield 88%; mp: 138-141°C; $^1$H-NMR: $\delta$ 8.12 (1H, d, J=9.0 Hz), 8.05 (1H, m), 8.02 (1H, d, J=2.4 Hz), 7.75 (1H, dd, J=9.0 Hz, J=2.4 Hz), 7.45 (1H, m), 7.28 (1H, m), 2.77 (3H, s), 2.76 (3H, s); EIMS m/z: 224 (M$^+$); HRMS (EI): C$_{13}$H$_{12}$N$_4$ Calculated: 224.1062; Found 224.1061.

2,3-di(2-furanyl)-6-(1-pyrrolidinyl)-quinoxaline (12): A yellow solid, yield 93%; mp: 180-182°C; $^1$H-NMR: $\delta$ 7.95 (1H, d, J=9.3 Hz), 7.58–7.62 (2H, m), 7.25 (1H, dd, J=2.7 Hz, J=9.3 Hz), 7.00 (1H, d, J=2.7 Hz), 6.44–6.56 (4H, m), 3.46–3.51 (4H, m), 2.06–2.13 (4H, m); EIMS m/z: 331 (M$^+$); HRMS (EI): C$_{20}$H$_{17}$N$_3$O$_2$ Calculated: 331.1321; Found 331.1306.

2,3-di(2-furanyl)-6-(1-piperidinyl)-quinoxaline (13): A yellow solid, yield 90%; mp: 111-113°C; $^1$H-NMR: $\delta$ 7.93 (1H, d, J=9.3 Hz), 7.57–7.60 (2H, m), 7.51 (1H, dd, J=2.7 Hz, J=9.3 Hz), 7.31 (1H, d, J=2.7 Hz), 6.46–6.56 (4H, m), 3.40–3.44 (4H, m), 1.66–1.73 (6H, m); EIMS m/z: 345 (M$^+$); HRMS (EI): C$_{21}$H$_{19}$N$_3$O$_2$ Calculated: 345.1477; Found 345.1497.

2,3-di(2-furanyl)-6-(1-azepanyl)-quinoxaline (14): A yellow solid, yield 73%; mp: 227-230°C; $^1$H-NMR: $\delta$ 7.92 (1H, d, J=9.6 Hz), 7.56–7.61 (2H, m), 7.35 (1H, dd, J=2.7 Hz, J=9.6 Hz), 7.12 (1H, d, J=3.0 Hz), 6.41–6.53 (4H, m), 3.61–3.65 (4H, m), 1.85–1.87 (4H, m), 1.56–1.61 (4H, m); EIMS m/z: 359 (M$^+$); HRMS (EI): C$_{22}$H$_{21}$N$_3$O$_2$ Calculated: 359.1634; Found 359.1649.

N-2,3-di(2-furanyl)-6-quinoxalinyl-4-(methoxy)-benzenemethanamine (15): A yellow solid, yield 80%; mp: 238-240°C; $^1$H-NMR: $\delta$ 7.87–7.90 (1H, m), 7.57–7.60 (2H, m), 7.31–7.34 (2H, m), 7.08–7.13
(2H, m), 6.89~6.92 (2H, m), 6.46~6.55 (4H, m), 4.40 (2H, s), 3.81 (3H, s); EIMS m/z: 397 (M⁺); HRMS (EI): C₂₄H₁₉N₃O₃ Calculated: 397.1426; Found 397.1422.

2,3-di(2-furanyl)-6-(1H-pyrrol-1-yl)-quinoxaline (16): A yellow solid, yield 94%; mp: 146-148°C; ¹H-NMR: δ 8.19 (1H, d, J=9.2 Hz), 8.10 (1H, d, J=2.4 Hz), 7.88 (1H, dd, J=9.2 Hz, J=2.4 Hz), 7.64~7.66 (2H, m), 7.30 (2H, m), 6.71 (1H, m), 6.66 (1H, m), 6.57~6.60 (2H, m), 6.44 (2H, m); EIMS m/z: 327 (M⁺); HRMS (EI): C₂₀H₁₃N₃O₂ Calculated: 327.0993.

2,3-di(2-furanyl)-6-(1H-imidazol-1-yl)-quinoxaline (17): A yellow solid, yield 97%; mp: 115-117°C; ¹H-NMR: δ 8.27 (1H, d, J=9.0 Hz), 8.16 (1H, d, J=2.7 Hz), 8.10 (1H, s), 7.85 (1H, dd, J=2.7 Hz, J=9.0 Hz), 7.66~7.68 (2H, m), 7.49 (1H, m), 7.31 (1H, m), 6.72~6.78 (2H, m), 6.60~6.63 (2H, m); EIMS m/z: 328 (M⁺); HRMS (EI): C₁₉H₁₂N₄O₂ Calculated: 328.0960; Found 328.0956.

2,3-di(2-furanyl)-6-(1H-pyrazol-1-yl)-quinoxaline (18): A yellow solid, yield 97%; mp: 171-173°C; ¹H-NMR: δ 8.38 (1H, dd, J=2.7 Hz, J=9.3 Hz), 7.35~7.41 (4H, m), 7.25 (1H, dd, J=9.3 Hz, J=2.7 Hz), 7.09~7.13 (4H, m), 6.99 (1H, d, J=2.7 Hz), 3.47 (4H, m), 3.35 (3H, s), 3.34 (3H, s), 2.09 (4H, m); EIMS m/z: 379 (M⁺); HRMS (EI): C₂₆H₂₅N₃ Calculated: 379.2049; Found 379.2048.

2,3-di(4-methylphenyl)-6-(1-pyrrolidinyl)-quinoxaline (19): A yellow solid, yield 90%; mp: 191-193°C; ¹H-NMR: δ 7.95 (1H, d, J=9.3 Hz), 7.35~7.41 (4H, m), 7.25 (1H, dd, J=9.3 Hz, J=2.7 Hz), 7.09~7.13 (4H, m), 6.99 (1H, d, J=2.7 Hz), 3.47 (4H, m), 3.35 (3H, s), 3.34 (3H, s), 2.09 (4H, m); EIMS m/z: 379 (M⁺); HRMS (EI): C₂₆H₂₅N₃ Calculated: 379.2049; Found 379.2048.

2,3-di(4-methylphenyl)-6-(1-azepanyl)-quinoxaline (20): A yellow solid, yield 50%; mp: 123-125°C; ¹H-NMR: δ 7.93 (1H, d, J=9.3 Hz), 7.33~7.39 (5H, m), 7.08~7.13 (5H, m), 3.62~3.66 (4H, m), 2.35 (3H, s), 2.34 (3H, s), 1.86~1.87 (4H, m), 1.56~1.61 (4H, m); EIMS m/z: 407 (M⁺); HRMS (EI): C₂₈H₂₉N₃ Calculated: 407.2361; Found 407.2360.

2,3-di(4-methylphenyl)-6-(1H-pyrrol-1-yl)-quinoxaline (21): A yellow solid, yield 96%; mp: 198-200°C; ¹H-NMR: δ 8.20 (1H, d, J=9.0 Hz), 8.11 (1H, d, J=2.4 Hz), 7.87 (1H, dd, J=2.4 Hz, J=9.0 Hz), 7.42~7.46 (4H, m), 7.30~7.32 (2H, m), 7.15~7.18 (4H, m), 6.44~6.46 (2H, m), 2.38 (6H, s); EIMS m/z: 375 (M⁺); HRMS (EI): C₂₆H₂₁N₃ Calculated: 375.1710; Found 375.1713.

2,3-di(4-methylphenyl)-6-(1H-pyrazol-1-yl)-quinoxaline (22): A yellow solid, yield 96%; mp: 218-220°C; ¹H-NMR: δ 8.31~8.36 (2H, m), 8.22~8.26 (1H, m), 8.15 (1H, m), 7.83 (1H, m), 7.44~7.47 (4H, m), 7.15~7.19 (4H, m), 6.58 (1H, m), 2.39 (6H, s); EIMS m/z: 376 (M⁺); HRMS (EI): C₂₅H₂₀N₄ Calculated: 376.1688; Found 376.1710.

**JSP-1 Inhibition Activities Assay**

The JSP-1 activity was determined at room temperature by monitoring the hydrolysis of 3-o-methylfluorescein phosphate (OMFP). In a typical assay a mixture (100 µL) containing 50 mM
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Bis·Tris, pH 6.5, 1 mM EDTA, 1 mM DTT, 10 µM OMFP and 30 nM purified recombinant GST-fusion human JSP-1 was used. The enzyme activity was continuously monitored with an excitation 485 nm/emission 535 nm filter set for 3 min and the initial rate of the hydrolysis was determined using the linear region of the enzymatic reaction kinetic curve. IC50 values were calculated from the non-linear curve fitting of percent inhibition (% inhibition) vs. inhibitor concentration [I] by using the equation: % Inhibition = 100/{1+(IC50/[I])k}, where k is the Hill coefficient.

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References

1. Nikam, S. S.; Cordon, J. J.; Ortwine, D. F.; Heimbach, T. H.; Blackburn, A. C.; Vartanian, M. G.; Nelson, C. B.; Schwarz, R. D.; Boxer, P. A.; Rafferty, M. F. Design and synthesis of novel quinoxaline-2,3-dione AMPA/GlyN receptor antagonists: Amino acid derivatives. J. Med. Chem. 1999, 42, 2266-2271.
2. Auberson, Y. P.; Bischoff, S.; Moretti, R.; Schmutz, M.; Veenstra, S. J. 5-Aminomethylquinoxaline-2, 3-diones. Part I: A novel class of AMPA receptor antagonists. Bioorg. Med. Chem. Lett. 1998, 8, 65-70.
3. Auberson, Y. P.; Acklin, P.; Allgeier, H.; Biollaz, M.; Bischoff, S.; Ofner, S.; Veenstra, S. J. 5-Aminomethylquinoxaline-2,3-diones. Part II: N-aryl derivatives as novel NMDA/glycine and AMPA antagonists. Bioorg. Med. Chem. Lett. 1998, 8, 71-74.
4. Kim, K. S.; Qian, L. G.; Dickinson, K. E. J.; Delaney, C. L.; Bird, J. E.; Waldron, T. L.; Moreland, S. Synthesis, biological properties, and structure-activity-relationships of quinoxaline angiotensin-II receptor antagonists. Bioorg. Med. Chem. Lett. 1993, 3, 2667-2670.
5. Kim, K. S.; Qian, L. G.; Bird, J. E.; Dickinson, K. E. J.; Moreland, S.; Schaeffer, T. R.; Waldron, T. L.; Delaney, C. L.; Weller, H. N.; Miller, A. V. Quinoxaline N-Oxide containing potent angiotensin-II receptor antagonists - synthesis, biological properties, and structure-activity-relationships. J. Med. Chem. 1993, 36, 2335-2342.
6. Perez-Melero, C.; Maya, A. B. S.; del Rey, B.; Pelayez, R.; Caballero, E.; Medarde, M. A new family of quinoline and quinoxaline analogues of combretastatins. Bioorg. Med. Chem. Lett. 2004, 14, 3771-3774.
7. Piras, S.; Loriga, M.; Paglietti, G. Quinoxaline chemistry. Part XVII. Methyl [4-(substituted 2-quinoxalinyloxy) phenyl] acetates and ethyl N-[(4-(substituted 2-quinoxalinyloxy) phenyl) acetyl] glutamates analogs of methotrexate: synthesis and evaluation of in vitro anticancer activity. Il Farmaco, 2004, 59, 185-194.
8. Corona, P.; Vitale, G.; Loriga, M.; Paglietti G. Quinoxaline chemistry. Part 13: 3-carboxy-2-benzylamino-substituted quinoxalines and N-[4-{(3-carboxyquinoxalin-2-yl) aminomethyl]benzoyl]-L-glutamates: synthesis and evaluation of in vitro anticancer activity. Il Farmaco, 2000, 55, 77-86.
9. Monge, A.; Palop, J. A.; de Cerain A. L.; Senador, V.; Martinez-Crespo, F. J.; Sainz, Y.; Narro, S.; Garcia, E.; de Miguel, C.; Gonzalez, M.; Hamilton, E.; Barker, A. J.; Clarke, E. D.; Greenhow, D. T. Hypoxia-selective agents derived from quinoxaline 1,4-di-N-oxides. *J. Med. Chem.* 1995, 38, 1786-1792.

10. Carta, A.; Loriga, M.; Zanetti, S.; Sechi, L. A. Quinoxalin-2-ones: Part 5. Synthesis and antimicrobial evaluation of 3-alkyl-, 3-halomethyl- and 3-carboxyethylquinoxaline-2-ones variously substituted on the benzo-moiety. *Il Farmaco* 2003, 58, 1251-1255.

11. Hui, X.; Desrivot, J.; Bories, C.; Loiseau, P. M.; Franck, X.; Hocquemiller, R.; Figadere, B. Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines. *Bioorg. Med. Chem. Lett.* 2006, 16, 815–820.

12. Alamin, R. J. Thiocyanatoquinoxaline compounds with immunomodulating activity. U.S. Patent 4,540,693, 1985; [Chem. Abstr. 1986, 104, 88601].

13. Li, J.; Chen, J.; Zhang, L.; Wang, F.; Gui, C.; Zhang, L.; Qin, Y.; Xu, Q.; Liu, H.; Nan, F.; Shen, J.; Bai, D.; Chen, K.; Shen, X.; Jiang, H. One novel quinoxaline derivative as a potent human cyclophilin A inhibitor shows highly inhibitory activity against mouse spleen cell proliferation. *Bioorg. Med. Chem.* 2006, 14, 5527–5534.

14. Shen, Y.; Luche, R.; Wei, B.; Gordon, M. L; Diltz, C. D.; Tonks, N. K. Activation of the Jnk signaling pathway by a dual-specificity phosphatase, JSP-1. *Proc. Natl. Acad. Sci U.S.A.* 2001, 98, 13613-13618.

15. Alonso, A.; Merlo, J. J.; Na, S. Q.; Kholod, N.; Jaroszewski, L.; Kharitonenkov, A.; Williams, S.; Godzik, A.; Posada, J. D.; Mustelin, T. Inhibition of T Cell Antigen Receptor Signaling by VHR-related MKPX (VHX), a New Dual Specificity Phosphatase Related to VH1 Related (VHR). *J. Biol. Chem.* 2002, 277, 5524–5528.

16. Chen, A. J.; Zhou, G. S.; Juan, T.; Colicos, S. M.; Cannon, J. P.; Cabrera-Hansen, M.; Meyer, C. F.; Jurecic, R.; Copeland, N. G.; Gilbert, D. J.; Jenkins, N. A.; Fletcher, F.; Tan, T. H.; Belmont, J. W. The dual specificity JKAP specifically activates the c-Jun N-terminal kinase pathway. *J. Biol. Chem.* 2002, 277, 36592-36601.

17. Alonso, A.; Rojas, A.; Godzik, A.; Mustelin, T. The dual-specific protein tyrosine phosphatase family. *Top. Curr. Genetics* 2004, 5, 333-358.

18. Davis, R. J. Signal transduction by the JNK group of MAP kinases. *Cell* 2000, 103, 239–252.

19. Manning, A. M.; Davis, R. J. Targeting JNK for Therapeutic Benefit: from JUNK to gold? *Nat. Rev. Drug Disc.* 2003, 2, 554-565.

20. Zhao, Z. J.; Wisnoski, D. D.; Wolkenberg, S. E.; Leister, W. H.; Wang, Y.; Lindsley, C. W. General microwave-assisted protocols for the expedient synthesis of quinoxalines and heterocyclic pyrazines. *Tetrahedron Lett.* 2004, 45, 4873-4876.

21. Abdel-Jalil, R. J.; Al-Qawasmeh, R. A.; Voelter, W.; Heeg, P.; El-Abadelah, M. M.; Sabri, S. S. Synthesis and properties of some 2,3-disubstituted 6-fluoro-7-(4-methyl-1-piperazinyl)quinoxalines. *J. Heterocycl. Chem.* 2000, 37, 1273-1275.

22. Charushin, V. N.; Mokrushina, G. A.; Tkachev, A. V. Nucleophilic substitutions in 6,7-difluoroquinoxalines. *J. Fluorine Chem.* 2001, 107, 71-80.
23. Kotovskaya, S. K.; Romanova, S. A.; Charushin, V. N.; Chupakhin, O. N. Fluorine-containing heterocycles: VII. Nucleophilic substitution in 6,7-difluoroquinoxalines. *Russ. J. Org. Chem.* **2002**, *38*, 1046-1052.

24. Takano, Y.; Shiga, F.; Asano, J.; Ando, N.; Uchiki, H.; Anraku, T. Synthesis and AMPA receptor antagonistic activity of a novel class of quinoxalinecarboxylic acid with a substituted phenyl group at the C-7 position. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3521–3526.

25. Takano, Y.; Shiga, F.; Asano, J.; Ando, N.; Uchiki, H.; Fukuchi, K.; Anraku, T. Design, synthesis, and AMPA receptor antagonistic activity of a novel 6-nitro-3-oxoquinoxaline-2-carboxylic acid with a substituted phenyl group at the 7 position. *Bioorg. Med. Chem.* **2005**, *13*, 5841-5863.

26. (a) Olsson, K.; Grivas, S. New synthetic routes to the potent mutagen 3,7,8-Trimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine. *Acta Chem. Scand. Ser.B.* **1986**, *40*, 486-492. (b) Grivas, S.; Olsson, K. An improved synthesis of 3,8-Dimethyl-3H-imidazo[4,5-f]quinoxalin-2-amine(“MeIQx”) and its 2-14C-labelled analogue. *Acta Chem. Scand. Ser.B.* **1985**, *39*, 31-34.

27. Kappe, C. O. Controlled microwave heating in modern organic synthesis. *Angew. Chem. Int. Ed.* **2004**, *43*, 6250-6284.

28. Ersmark, K.; Larhed, M.; Wannberg, J. Microwave-enhanced medicinal chemistry: A high-speed opportunity for convenient preparation of protease inhibitors. *Curr. Opin. Drug Discov. Dev.* **2004**, *7*, 417-427.

29. Mavandadi, F.; Lidstrom, P. Microwave - Assisted chemistry in drug discovery. *Curr. Top. Med. Chem.* **2004**, *4*, 773-792.

30. Lidstrom, P.; Tierney, J.; Wathey, B.; Westman, J. Microwave assisted organic synthesis–a review. *Tetrahedron* **2001**, *57*, 9225-9283.

31. Xu, Y.; Guo, Q. X. Syntheses of heterocyclic compounds under microwave irradiation. *Heterocycles*. **2004**, *63*, 903-974.

32. Wilson, N. S.; Roth, G. P. Recent trends in microwave-assisted synthesis. *Curr. Opin. Drug Discov. Dev.* **2002**, *5*, 620-629.

33. Ding, D. R.; Li, X.; Wang, X.; Du, Y. L.; Shen, J. K. Microwave-assisted rapid and straightforward synthesis of 2-aryl-4-quinolones from acylated 2'-aminoacetophenones. *Tetrahedron Lett.* **2006**, *47*, 6997-6999.

34. Ahmad, A.; Dunbar, L. J.; Green, I. G.; Harvey, I. W.; Shepherd, T.; Smith, D. M.; Wong, R. K. C. Polyaza heterocycles. Part 2. Nucleophilic substitution of halogens in halogenoquinoxalino[2,3-c]cinnolines. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2751-2758.

35. Cutshall, N. S.; O’Day, C.; Prezhdo, M. Rhodanine derivatives as inhibitors of JSP-1. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 3374-3379.

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