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# Quinoxalinylurea derivatives as a novel class of JSP-1 inhibitors

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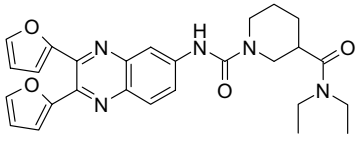
**Abstract**—A series of quinoxalinylurea-based inhibitors are synthesized and shown to be the novel and potent inhibitors against Jnk Stimulatory Phosphatase-1 (JSP-1), which is a special member of dual-specificity protein phosphatase (DSP) family. Biological assay and computational modeling studies showed the compounds were reversible and noncompetitive inhibitors of JSP-1. JSP-1 inhibitors may be useful for the treatment of inflammatory, vascular, neurodegenerative, metabolic, and oncological diseases in humans associated with dysfunctional Jnk signaling.


JSP-1 (Jnk Stimulatory Phosphatase-1),[1] also referred to as VHX (VHR-related MKPX)[2] or JKAP (Jnk pathway-associated phosphatase),[3] is a special member of the dual-specificity protein phosphatases (DSPs),[4] which belong to the protein tyrosine phosphatases (PTPs),[5] a super-family of enzymes with similarly conserved motif HCXXGXXR and the same tertiary structure. PTPs dephosphorylate proteins with phosphate on serine, threonine, and/or tyrosine, and play important regulative roles in cellular signal transduction. In recent years, interest in PTPs as potential drug targets for several serious diseases such as cancers, autoimmune diseases, and diabetes has rapidly increased.[6,7] While some members of DSPs like VHR (human VH1-related) and MKP1 are negative regulators of mitogen activated protein kinases (MAPK), JSP-1 is a positive regulator for the Jun $NH_2$-terminal kinase (Jnk) pathway. The study of Belmont's group[3] indicated that JSP-1 is necessary for optimal Jnk activation. However, JSP-1 does not exert its effects directly on Jnk, it appears to work upstream of Jnk itself, by activating MKK4[1] and MKK7[3] kinases, which phosphorylate and activate Jnk.

The Jnk pathway plays broad roles in cellular response to various forms of stresses, growth stimulation, and apoptosis.[8] Dysfunctional Jnk signaling is associated with inflammatory, vascular, neurodegenerative, metabolic, and oncological diseases in humans.[9] Therefore, effect of JSP-1 on the Jnk signal pathway makes it worth studying as a potential novel therapeutic target.

To date, reports regarding small molecule JSP-1 inhibitors are few.[10] Through a high-throughput screening of our sample collection, a quinoxalinylurea-based small molecule compound **A1** (Fig. 1) was discovered and showed inhibiting activity toward JSP-1 with the 50% inhibitory concentration ($IC_{50}$) of $12.01 \pm 0.15$ μM in an in vitro biological assay.

Quinoxalines are an important class of nitrogen-containing heterocycles with antibacterial activity,[11,12] angiotensin II receptor,[13,14] and AMPA receptor antagonist activity.[15,16] In this paper, a series of quinoxali-



**A1** $IC_{50} = 12.01 \pm 0.15$μM

**Figure 1.** Inhibitor of JSP-1 discovered by a high-throughput screening.

*Keywords*: JSP-1; Inhibitor; Quinoxalinylurea.
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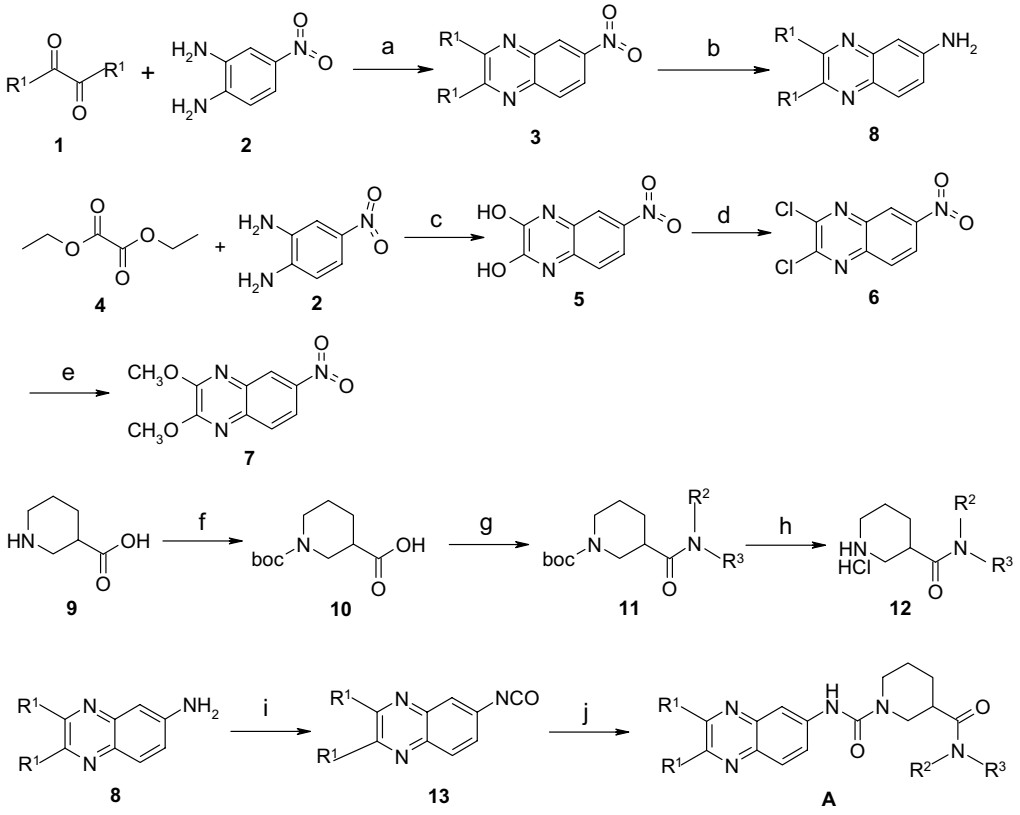
nylurea-based small molecules are reported as novel and potent JSP-1 inhibitors.

With the goal to investigate the structure–activity relationship (SAR) of these quinoxalinylurea-based small molecules, a library with 28 of these analogs were designed and synthesized. Keeping the *N*-6-quinoxalinyl-1,3-piperidinedicarboxamide moiety, aryl and alternative lipophilic groups such as phenyl, 4-methylphenyl, 4-fluorophenyl, 4-bromophenyl, and methoxyl were employed as replacements for the furyl groups in the 2 and 3 positions on the quinoxaline ring, and cyclic primary and secondary amines such as pyrrolidine, piperidine, cyclopentylamine, and cyclohexylamine were used to displace diethylamide of **A1** in an effort to investigate whether these groups were essential for retaining the activity in this series.

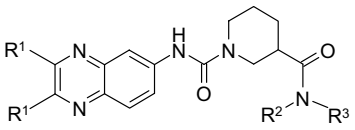
The synthetic routes of these compounds described in this study are outlined in Scheme 1. In general, 2,3-disubstituted 6-nitro-quinoxaline **3** was obtained by the condensation of 1,2-dicarbonyl compound **1** with 4-nitro-*o*-phenylenediamine **2**.[17,18] 2,3-dimethoxy-substituted analog of 6-nitro-quinoxaline **7** was synthesized by the condensation of diethyl oxalate **4** with 4-nitro-*o*-phenylenediamine **2**, followed by the chlorination with $POCl_3$, and then the substitution with $CH_3ONa$.[19,20] The catalytic hydrogenation of 6-nitro-quinoxaline **3** and **7** in the presence of 10% Pd/C in EtOH afforded the corresponding 6-aminoquinoxaline derivatives **8**, which were treated with triphosgene to provide crude 2,3-disubstituted-6-isocyanato-quinoxalines derivatives **13**[21] to be used in the next reaction without further purification. On the other hand, piperidine-3-carboxylic acids **9** were treated with $(Boc)_2O$ to give Boc-protected piperidine-3-carboxylic acids **10**, which were coupled with various amines in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxy benzotriazole (HOBt) to afford Boc-protected piperidine-3-amides **11**. After removing Boc-protecting group, the corresponding piperidine-3-amide hydrochlorides **12** were produced. Finally, the designed compounds were obtained by the reaction of **13** with piperidine-3-amides **12**.

All synthesized compounds were screened for their ability to inhibit JSP-1 in an in vitro enzymatic assay.[22] A part of biological results with representative structures are listed in Table 1, and the other compounds whose $IC_{50}$ exceed 40 μM were not described in detail. The results showed that substitution at positions 2 and 3 on the quinoxaline ring plays an important role in the inhibitory activity. Keeping the furyl groups at positions 2 and 3, compounds (**A2**–**A4**) showed potent inhibition against JSP-1. Replacements of the furyl groups in positions 2 and 3 of the quinoxaline ring with phenyl, or substituted phenyl groups resulted in decreased potency (**A5**–**A12**), while replacement with the lipophilic methoxyl




**Scheme 1.** Reagents and conditions: (a) EtOH, reflux, 24 h (90–96%); (b) $H_2$, 10% Pd/C, EtOH, rt, 3 h (90–96%); (c) reflux, 24 h (89%); (d) $POCl_3$, reflux, 3.5 h (66%); (e) $CH_3ONa$, $CH_3OH$, rt, 3 h (91%); (f) $(Boc)_2O$ (98%); (g) $HNR^2R^3$, EDC, HOBt, $CH_2Cl_2$, rt (95%); (h) HCl in dioxane (90–95%); (i) triphosgene, DIPEA, $CH_2Cl_2$, rt; (j) 12, DIPEA, $CH_2Cl_2$, rt.

**Table 1.** Activities of quinoxalinylurea derivatives



| Compound | $R^1$ | $NR^2R^3$ | JSP-1 $IC_{50}$ (μM)[a] |
|---|---|---|---|
| **A1** | 2-Furyl | Diethylamine | 12.01 ± 0.15 |
| **A2** | 2-Furyl | Piperidine | 8.39 ± 1.50 |
| **A3** | 2-Furyl | Cyclopentylamine | 3.02 ± 0.18 |
| **A4** | 2-Furyl | Cyclohexylamine | 2.25 ± 0.24 |
| **A5** | Phenyl | Diethylamine | >40 |
| **A6** | Phenyl | Piperidine | >40 |
| **A7** | 4-Methylphenyl | Diethylamine | >40 |
| **A8** | 4-Methylphenyl | Cyclopentylamine | >40 |
| **A9** | 4-Fluorophenyl | Diethylamine | >40 |
| **A10** | 4-Fluorophenyl | Cyclohexylamine | >40 |
| **A11** | 4-Bromophenyl | Diethylamine | >40 |
| **A12** | 4-Bromophenyl | Pyrrolidine | >40 |
| **A13** | Methoxy | Piperidine | >100 |
| **A14** | Methoxy | Cyclohexylamine | >100 |

[a] Data are means of three independent experiments.

group was not tolerated (**A13**–**A14**). In this series, compound **A4** showed the most potent inhibition of JSP-1. Detailed enzymatic kinetics studies revealed that compound **A4** is a reversible and noncompetitive inhibitor of JSP-1 (Figs. 2 and 3),[22,23] suggesting that this compound may utilize an allosteric mechanism to inhibit JSP-1.

As mentioned by Wiesmann et al.,[24] when an inhibitor is bound to the allosteric modulation pocket of protein tyrosine phosphatase-1B (PTP1B), it could lock the PTP1B at the inactive conformation. This could be compared to our case with JSP-1. Although three-dimentional structure of JSP-1 was solved by Yakota et al., (PDB access code 1WRM),[25] we are aware that JSP-1 in the crystal structure is remaining in an active conformation. Through structure and sequence alignments, the catalytic domain of the inactive conformation of MAPK

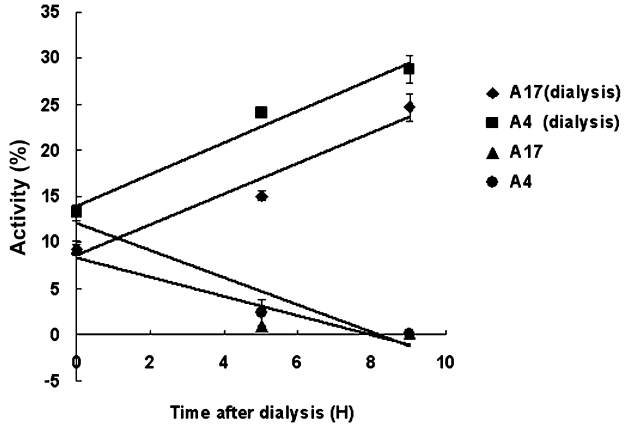



**Figure 2.** Reversible inhibitors of JSP-1.

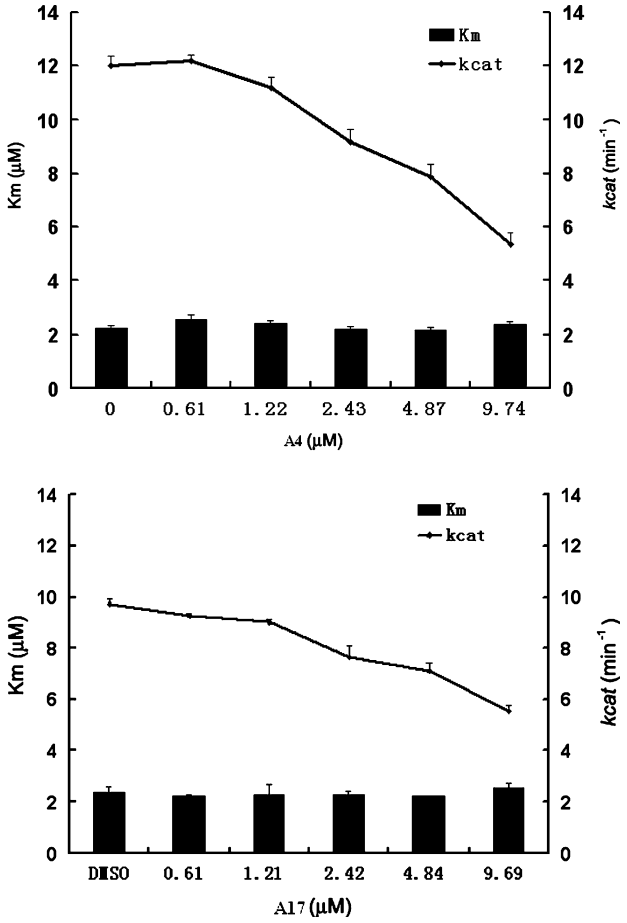



**Figure 3.** Noncompetitive inhibitors of JSP-1.

phosphatase Pyst1 (PDB access code 1MKP) was utilized as a structure template to model the inactive conformation of JSP-1. The allosteric binding site was indicated by the superimposed structure of PTP1B (PDB access code 1T48), which contained helix α3 (residues 65–80) and helix α6 (residues 137–152) in JSP-1. The advanced docking software AUTODOCK3.0 was used to dock the **A4** into this binding pocket (Fig. 4).[26] As suggested by the docking study, there are several hydrophobic residues close to the piperidine-3-carboxamide and this binding interaction provided evidence that the large side chain group on the piperidine-3-carboxamide may improve inhibiting JSP-1 activity.

To corroborate the hypothesis coming from the docking study, a series of 2,3-difuryl quinoxalinyl ureas incorporating various bulky alkyl amines shown in Table 2 were designed for the second round. Difuryl substitutes at positions 2 and 3 of the quinoxaline ring were retained and alternative substitutes on the piperidine carboxamide were explored. The compounds **A15**–**A23** were prepared by the same method as described above. As listed in Table 2, the results of the in vitro enzymatic assay showed that activity was influenced by the substituents at the piperidine-3-carboxamide. Replacement of the diethyl group with small alkyl groups resulted in reducing JSP-1 inhibitory activity (**A15**, **A19**–**A20**).

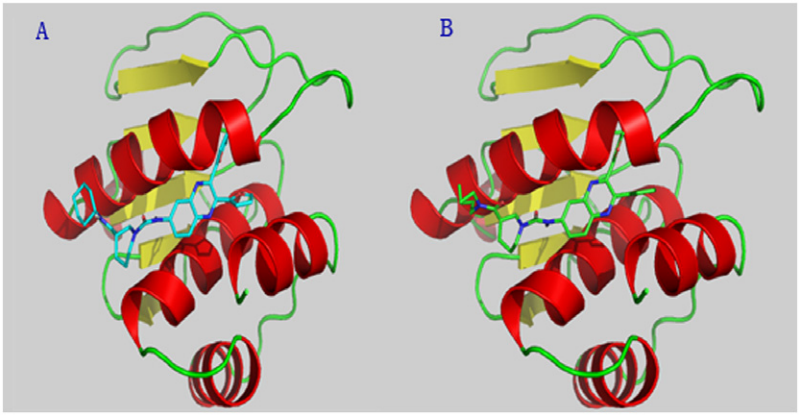
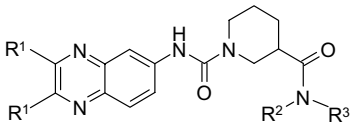



**Figure 4.** (A) View of allosteric site in presence of compound **A4**. (B) View of allosteric site in presence of compound **A17**.

**Table 2.** Activities of quinoxalinylurea derivatives



| Compound | $R^1$ | $NR^2R^3$ | JSP-1 $IC_{50}$ (μM)[a] |
|---|---|---|---|
| **A15** | 2-Furyl | Dimethylamine | >40 |
| **A16** | 2-Furyl | *N*-Methyl-butylamine | 5.55 ± 0.56 |
| **A17** | 2-Furyl | *N*-Ethyl-butylamine | 2.35 ± 0.65 |
| **A18** | 2-Furyl | Dipropylamine | 2.50 ± 0.06 |
| **A19** | 2-Furyl | Ethylamine | >40 |
| **A20** | 2-Furyl | Isopropylamine | >40 |
| **A21** | 2-Furyl | *tert*-Butylamine | 8.05 ± 0.97 |
| **A22** | 2-Furyl | Isobutylamine | 8.36 ± 0.18 |
| **A23** | 2-Furyl | *n*-Butylamine | 2.79 ± 0.25 |

[a] Data are means of three independent experiments.

Replacing with long chain or sterically hindered alkyl groups, the compounds **A16**–**A18** and **A21**–**A23** showed significant activity comparable with **A2**–**A4**. Furthermore, enzymatic kinetics studies on compound **A17** also indicated that it is a reversible and noncompetitive inhibitor of JSP-1 (Figs. 2 and 3).

In summary, we have synthesized and investigated preliminary SAR for a novel series of quinoxalinylurea derivatives with JSP-1 inhibitory activity based on the compound **A1** obtained through high-throughput screening. The result of in vitro biological experiment showed that these compounds were noncompetitive and reversible inhibitors of JSP-1. Through computational modeling, an allosteric site in JSP-1 was found, and based on the docking study we provided a hypothesis that a hydrophobic subpocket is close-by and can be utilized to improve the binding affinity. The biological assay performed on the second round compounds confirmed that bulky alkyl groups appended to the piperidine-3-carboxamide would benefit the affinity to JSP-1. The further study is going on.

## Acknowledgment

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22. The JSP-1 activity was determined at room temperature by monitoring the hydrolysis of 3-*o*-methylfluorescein phosphate (OMFP). The studies have shown that OMFP is an ideal substrate for dual-specificity phosphatases as they evoke faster kinetics for hydrolysis of OMFP compared with pNPP. In a typical 100 μL assay mixture containing 50 mM Bis·Tris, pH 6.5, 1 mM EDTA, 1 mM DTT, 10 μM OMFP, and 30 nM purified recombinant GST-fusion human JSP-1, the enzyme activity was continuously monitored with 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. $IC_{50}$ was calculated from the nonlinear curve fitting of percent inhibition (% inhibition) versus inhibitor concentration [I] by using the following equation% Inhibition = $100/\{1 + (IC_{50}/[I])k\}$, where $k$ is the Hill coefficient. For mechanism studies, data were analyzed using a nonlinear regression fit according to classical Michaelis–Menten kinetics models.
23. The reversibility of inhibition is easily determined by measuring the recovery of enzymatic activity after dialysis of the enzyme–inhibitor complex against assays. To test for reversibility, excessive compounds **A4** and **A17** were incubated with 100 nM enzyme. Then the mixture was dialyzed against the assay buffer and the activity of the enzyme was tested in different times. If the inhibitor was reversible, enzymatic activity could recover to some extent; and if the inhibitor was irreversible, enzymatic activity could not recover at any time. Figure 2 shows the activity of JSP-1 was recovered to some extent after dialysis, compared with the mixture without dialysis. It verified that compounds **A4** and **A17** were reversible JSP-1 inhibitors.
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