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# Rhodanine derivatives as inhibitors of JSP-1

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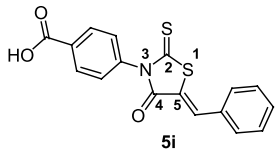
**Abstract**—Dual-specificity phosphatases (DSPs) are a subclass within the protein tyrosine phosphatase family (PTPs). A series of rhodanine-based inhibitors was synthesized and shown to be novel, potent, and selective inhibitors against the DSP family member JNK-stimulating phosphatase-1 (JSP-1). Compounds of this class may be useful for the treatment of inflammatory and proliferative disorders.


Protein tyrosine phosphatases (PTPs) are enzymes that regulate and control cellular processes including growth, proliferation, differentiation, metabolism, the immune response, cell–cell adhesion, and cell–matrix contacts.[1] They are typified by the conserved active-site motif $CX_5R$. Inhibition of PTPs has been an active area of drug discovery.[2] Dual-specificity phosphatases (DSPs) are a subclass of PTPs that are named because of their unique ability to hydrolyze the phosphate ester bond on both a tyrosine residue and either a serine or threonine residue located in the same protein; the prototype being the MKP proteins. JSP-1 belongs to a subclass of 'atypical' DSPs that are noted for their small size but whose substrate specificity has not been elucidated. JSP-1 is also referred to as DUSP22, VHX, MKPX, LMW-DSP2, TS-DSP2, and JKAP, and is an attractive target because it may offer a novel therapeutic target for the treatment of various inflammatory and proliferative disorders associated with dysfunctional Jnk signaling.[1,3]

To date, no drug-like small molecules have been reported to inhibit the dual-specificity phosphatase JSP-1. In an effort to identify inhibitors of JSP-1, a high-throughput screening campaign was conducted and provided screening hits which contained a 5-benzylidene-3-phenyl-2-thioxo-thiazolidin-4-one (rhodanine) core and typified by compound **5i** (Fig. 1). Rhodanine-based molecules have been popular as small molecule inhibitors of numerous targets such as HCV NS3 protease,[4a] aldose reductase,[4b,c] β-lactamase,[4d] UDP-*N*-acetylmuramate/L-alanine ligase,[4e] antidiabetic agents,[4f] cathepsin D,[4g] and histidine decarboxylase.[4h] Here, we report the results of a novel class of rhodanine-based small molecules that inhibit JSP-1 in the low micromolar range.

Two routes were explored toward the synthesis of rhodanines (Scheme 1). In route A, the desired phenyl- or benzyl-isocyanate **1** was treated with methyl thioglycolate in the presence of triethylamine to provide the desired rhodanine intermediate **2**. Initially an excess of the scavenger resin *N*-(2-aminoethyl)aminomethyl polystyrene was used to conveniently remove any unreacted isocyanate but was later found to be unnecessary as intermediate **2** was readily purified via crystallization.[5] Intermediate **2** was readily solubilized in hot acetic acid and treated with concentrated HCl to effect hydrolysis of the ester functionality to provide **3**. A number of conditions are reported to effectively condense rhodanines with aromatic aldehydes.[4a,b] In our hands, it was found that treating **3** with the desired aldehyde using acetic acid and sodium acetate provided the 5-benzylidene-substituted rhodanines **5**, typically in >70% yield.[4g] In all cases, the thermodynamically stable *Z*-isomer predominated with a ratio of *Z*:*E* isomers $\geqslant$10:1 after




**5i**

$IC_{50}$ 18 μM

**Figure 1.** Competitive inhibitor of JSP-1.

*Keywords*: Rhodanine; PTP; DSP.
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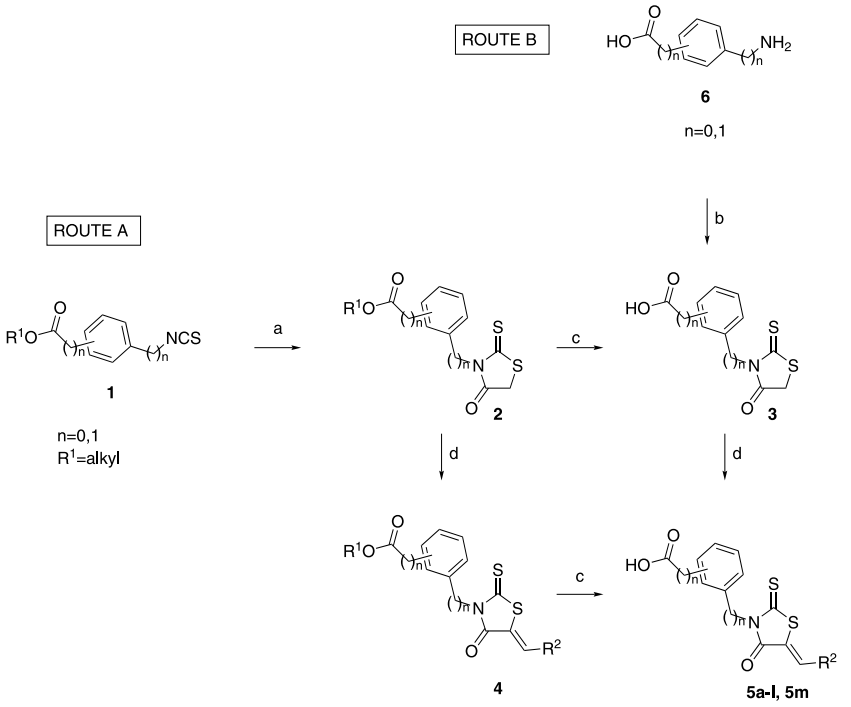


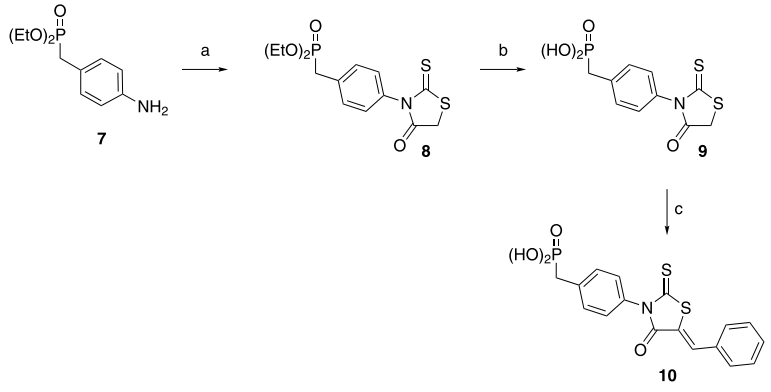

**Scheme 1.** Reagents and conditions: (a) methyl thioglycolate, $NEt_3$, $CH_2Cl_2$, rt; (b) bis(carboxymethyl)trithiocarbonate, sodium carbonate, water; (c) HCl/HOAc (1:1), reflux; (d) $R^2$–CHO, sodium acetate, HOAc.

recrystallization. The ratio of the two geometrical stereoisomers was readily quantified using $^1H$ NMR as previously reported in the literature.[4a,5] Alternatively, as shown in route A, ester-intermediate **2** could be condensed first with the desired aldehyde to provide **4** and then subsequently converted to **5** using the hydrolytic conditions previously described.

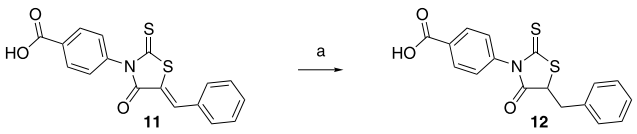
Due to the limited commercial availability of diverse isothiocyanates, a second synthetic strategy was employed for rhodanine synthesis (route B). Using this alternative method a wide variety of anilines or amines **6** could be treated with bis(carboxymethyl)trithiocarbonate in the presence of sodium carbonate to afford the desired rhodanine heterocycle **3**.[6] Condensation of **3** with aldehydes or ketones could then be accomplished as previously outlined (route A).

Scheme 2 outlines the synthetic route used to prepare compound **10**. Treatment of diethyl (4-aminobenzyl)-phosphonate with bis(carboxymethyl)-trithiocarbonate provided rhodanine **8**. Conversion of **8** to the corresponding phosphonic acid **9** was accomplished using bromotrimethylsilane under standard conditions. Treatment of **9** using conditions previously described readily provided **10**.

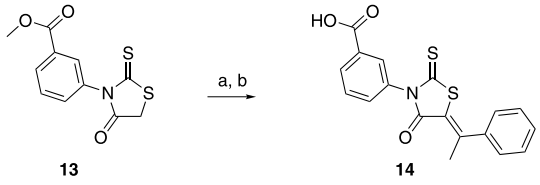
Modification of the exocyclic 5-benzylidene double bond was of interest for SAR studies (Schemes 3 and 4). Reduction of **11** was ultimately accomplished using lithium borohydride in pyridine followed by heating to provide **12** in 26% yield.[7] In contrast to literature reports, efforts to reduce **11** with sodium borohydride in DMF at 0 °C failed to produce any of the desired product.[8] Condensation of **13** with excess acetophenone and




**Scheme 2.** Reagents and conditions: (a) bis(carboxymethyl)-trithiocarbonate, sodium carbonate, water; (b) bromotrimethylsilane, $CH_2Cl_2$, rt; (c) PhCHO, sodium acetate, HOAc, 110–130 °C.




**Scheme 3.** Reagents: (a) $LiBH_4$, pyridine, THF.




**Scheme 4.** Reagents and conditions: (a) acetophenone, ammonium acetate, toluene, reflux; (b) HCl/HOAc (1:1), reflux.

ammonium acetate provided the corresponding alkene in a 1:10 ratio of geometrical isomers by $^1$H NMR. Based on literature precedence the isomeric ratio was presumed to be mainly the *Z* stereoisomer.[4a,5] Hydrolysis of the ester bond then provided **14** in quantitative yield and also with a 1:10 isomeric ratio.

The azolid-4-one analogs in Table 3 were made using the following referenced procedures: hydantoins **15b** and **c**,[9] 2-thioxo-4-oxazolidinone **15d**,[10] 2,4-thiazolidinedione **15e**.[11]

As part of a lead optimization strategy, rhodanines were screened in an in vitro enzymatic assay for their ability to inhibit JSP-1[12] while maintaining selectivity against VH1-related (VHR) phosphatase.

Structure–activity relationship (SAR) studies focused primarily on modifying three regions of the inhibitor: N-phenyl modifications, exploration of benzylidene phenyl substitution, and replacement of the rhodanine core heteroatoms.

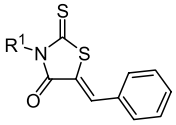
Initial SAR studies focused on modification of the 3-position of the rhodanine core (Table 1). It was determined early in the course of SAR studies that an acidic moiety, preferentially linked to an aryl substituent, was necessary for maintaining inhibition against JSP-1. It was hypothesized that the acidic moiety may function as a phenylphosphate mimetic since the physiological role of JSP-1 is to hydrolyze *p*Y or *p*T/S residues. Previous surveys of *p*Tyr replacements that target tyrosine kinase SH2 domains and PTPs have been recently reported.[13a–d] Based on this hypothesis we undertook a survey of potential phenylphosphate mimetics. Several trends in the SAR were noticed. We found that an aryl carboxylate at the meta position provided compounds that were approximately three times more potent than when the carboxylate was positioned in the *para* position as seen in compounds **5m** and **i**, respectively. Sulfonic and phosphonic acid containing compounds **5l** and **10** also provided modest potencies. Substitution at the ortho-position of the phenyl ring was not tolerated, possibly due to restricted rotation around the C–N bond[14] and led to significantly less potent compounds as seen with **5c** and **e**. Substituents not containing acidic functionalities completely failed to inhibit JSP-1 as demonstrated by compounds **5a** and **b**. With the exception of compound **5f**, all of the inhibitors maintained a Hill slope $>-1.3$ suggesting a stoichiometric relationship between enzyme and inhibitor. Additionally, all of the compounds showed remarkable selectivity against a panel of phosphatases including VHR, were competitive for substrate, and reversible when tested in a dilution assay (results not shown). Although, the biochemical profile showed that the inhibitors were reversible and displayed competitive kinetics, it could not be definitively concluded without an X-ray structure of the protein–inhibitor complex that the acidic moiety was serving as a phosphate mimetic.

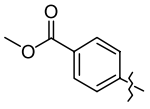
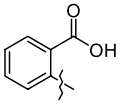
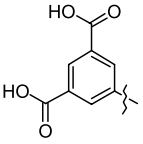
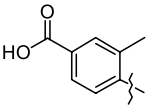
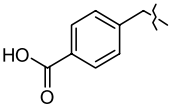
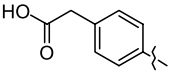
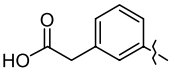
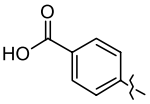
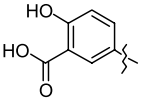
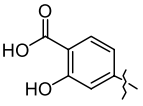
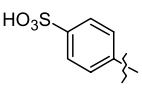
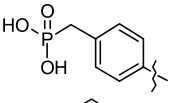
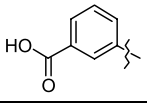
The second area of SAR efforts focused on modifications of the benzylidene functionality at the rhodanine 5-position. Table 2 shows a representative example of the substituents explored. Several distinct SAR trends were observed. All efforts to append a carboxylate functionality anywhere proximal to the benzylidene region resulted in a complete loss of activity as typified by compound **5n**. Modest potencies were observed with aryl functionalities that contained relatively weak electron-withdrawing to electron-donating aryl-substituents such as **5o**, **q**, and **r** as well as with alkyl substitutions such as **5p**.[15] In contrast, analogs that incorporated stronger electron-withdrawing aryl groups provided the most inhibitory compounds as illustrated by the 4-nitro- and 4-fluorophenyl analogs **5u** and **v**, respectively.

Since there was a correlation between the inhibitory activity and the class of molecules containing strongly electron withdrawing substituents on the benzylidene aryl ring, we speculated that a possible mechanism of inhibition could be due to a reversible Michael-type addition of a nucleophilic cysteine residue near the active site of JSP-1 to the rhodanine exocyclic double bond. This is consistent with previous reports that nucleophiles such as substituted thiophenols and piperidine are able to add in a reversible fashion to the exocyclic double bonds of rhodanines.[16] To test this hypothesis, several cysteine to alanine JSP-1 constructs were made and tested. It was in fact demonstrated through point specific mutation of JSP-1 that a cysteine residue near the active site was necessary for maintaining the inhibitory profile of the rhodanines (results not shown).[17] An inhibitory mechanism that relies on a covalent-reversible Michael-type addition of a nucleophilic protein residue with the inhibitor is also consistent with other SAR efforts that focused on either the removal or the benzylidene double bond or increasing its steric bulk. Indeed, it was found that reduction of the double bond negated JSP-1 inhibitory activity as did substitution of the exocyclic alkene with a methyl group (Schemes 3 and 4).

The final area of SAR explored changes to the rhodanine core (Table 3). As can be seen, inhibition of JSP-1 was only successful with rhodanines and any modification of the core heteroatoms led to analogs that

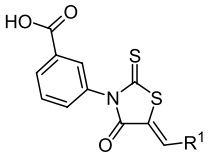
**Table 1.** Representative activities of 3-substituted rhodanines

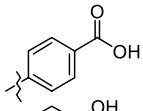
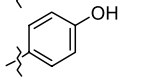
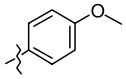
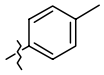
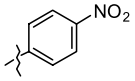
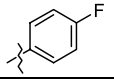


| Compound | Route | $R^1$ | JSP-1 $IC_{50}$ (μM)[a] | JSP-1 Hill slope[a,b] | VHR $IC_{50}$ (μM)[a] |
|---|---|---|---|---|---|
| **5a** | A |  | >200 | n.d. | n.d. |
| **5b** | A |  | >200 | n.d. | >n.d. |
| **5c** | A |  | >200 | n.d. | >200 |
| **5d** | B |  | 103 | n.d. | >200 |
| **5e** | B |  | 66 | −1.2 | >200 |
| **5f** | B |  | 60 | −1.6 | >200 |
| **5g** | B |  | 20 | −1.2 | 100 |
| **5h** | B |  | 20 | −1.1 | >200 |
| **5i** | A |  | 18 | −1.2 | >200 |
| **5j** | A |  | 13 | −1.1 | 130 |
| **5k** | A |  | 13 | −1.3 | 45 |
| **5l** | A |  | 11 | −1.2 | >200 |
| **10** | n.a. |  | 10 | −1.0 | >200 |
| **5m** | A |  | 6.6 | −0.9 | >100 |

[a] Values were calculated from the average of at least two experiments.
[b] n.d.=not determined.

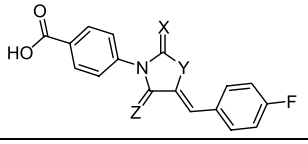
**Table 2.** Representative activities of rhodanines



| Compound | $R^1$ | JSP-1 $IC_{50}$ (μM)[a] | JSP-1 Hill slope[a] | VHR $IC_{50}$ (μM)[a] |
|---|---|---|---|---|
| **5n** |  | >200 | n.d. | >200 |
| **5o** |  | 157 | −1.3 | >200 |
| **5p** |  | 59 | −1.0 | >200 |
| **5q** |  | 26 | −1.8 | >200 |
| **5r** |  | 16 | −1.3 | 123 |
| **5s** |  | 7.3 | −1.1 | >200 |
| **5t** |  | 7 | −0.9 | >100 |
| **5u** |  | 2.6 | −0.9 | >50 |
| **5v** |  | 1.3 | −1.3 | >50 |

[a] Values were calculated from the average of at least two experiments.

**Table 3.** Replacement of rhodanine core heteroatoms



| Compound | X | Y | Z | JSP-1 $IC_{50}$ (μM)[a] |
|---|---|---|---|---|
| **15a** | S | S | O | 4.2 |
| **15b** | S | NH | O | >200 |
| **15c** | S | NMe | O | >200 |
| **15d** | S | O | O | >200 |
| **15e** | O | S | O | >200 |

[a] Values were calculated from the average of at least two experiments.

failed to inhibit JSP-1. Based on these observations only a limited number of non-rhodanine analogs was made and tested.

In conclusion, the first reported synthesis and evaluation of rhodanine-based inhibitors of JSP-1 have been described. SAR studies demonstrated that stronger electron-withdrawing functional groups appended to the aryl-benzylidene position provided analogs with the greatest potencies as illustrated by compound **5v**. Compound **5v** was also reversible and competitive with substrate and showed a high degree of enzyme selectivity against other phosphatases. Ongoing studies will soon be reported which will help further elucidate the inhibitory mechanism of this class of inhibitors.[17]

## Acknowledgment

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$KH_2PO_4{\cdot}H_2O$. The slurry was centrifuged and half of the supernatant was counted in a Wallac Microbeta counter to determine activity using Cherenkov counting. Compounds were tested similarly on VHR with the exception that VHR was assayed at 290 ng/mL and phosphorylated RCML as a substrate at 0.1 μM. RCML was prepared as described in Ref. 16.

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