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SYNTHESIS OF NH-ACYL-α-AMINOAMIDES ON RINK RESIN: INHIBITORS OF THE HEMATOPOIETIC PROTEIN TYROSINE PHOSPHATASE (HePTP)

Xiaodong Cao, Edmund J. Moran, Daniel Siev[†], Anna Lio, Cara Ohashi, Adnan M. M. Mjalli*

Ontogen Corporation, 2325 Camino Vida Roble, Carlsbad, CA 92009

Abstract: Cinnamic acid derivatives were prepared on Rink resin using *tert*-butyl-4-carboxycinnamate in a four-component Ugi condensation. Inhibition of the hematopoietic protein tyrosine phosphatase (HePTP) by these small molecules is reported. An IC_{50} value of 3.9 μM was determined for the most potent inhibitor discovered.

Protein tyrosine phosphorylation by protein tyrosine kinases (PTKs) and dephosphorylation by protein tyrosine phosphatases (PTPases) have been shown to play a crucial role in the signal transduction pathways including those which control cell growth and differentiation.[1,2] Consistent with this role, aberrant levels of expression or function of PTPases have been involved in several disease areas such as cancer,[3] insulin dependent diabetes[4] and platelet aggregation response.[5] Hematopoietic protein tyrosine phosphatase (HePTP) is an intracellular, hematopoietic cell specific, single domain PTPase comprised of 339 amino acids.[6] Overexpression of HePTP has been implicated in acute leukemia.[7]

All PTPases share a highly conserved catalytic domain of eleven amino acids: (L/V)HCXAGXXR(S/T)G.[8] Site-directed mutagenesis[9] and chemical labeling[10] experiments showed that the cysteine residue is required for PTPase catalytic activity. Mechanistically, protein dephosphorylation proceeds via the transient formation of a covalent thiophosphate linkage between the cysteine moiety in the enzyme active site and the phosphate group of tyrosine.[11]

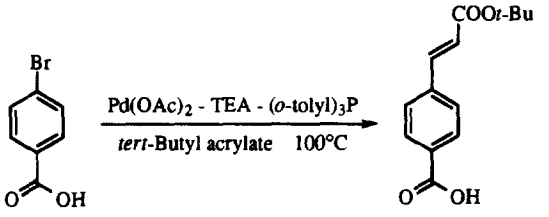
Although the physiologically relevant substrates for HePTP have yet to be identified, assays employing artificial substrates such as *para*-nitrophenyl phosphate (*p*-NPP) [12] have lead to the discovery of several inhibitors of PTPases. Peptides bearing non-hydrolyzable phosphotyrosyl mimetics have been

[†] Corvas International, Inc., 3030 Science Park Road, San Diego, California 92121

described as inhibitors of the PTP-1B dependent insulin receptor dephosphorylation.[13] Additionally, metal-containing, highly charged and peptide-based molecules have been reported as inhibitors of PTPases.[14] Herein, the synthesis of NH-acyl-α-aminoamides on Rink resin and the discovery of cinnamic acid derivatives **1a-1r** as novel non-phosphorous based inhibitors of HePTP are reported.

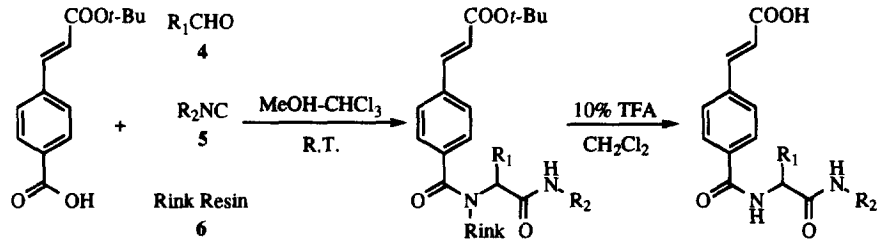
Chemistry

tert-Butyl-4-carboxycinnamate (**3**)[15] was synthesized by the reaction of 4-bromobenzoic acid (**2**) with *tert*-butyl acrylate in the presence of $Pd(OAc)_2$ in greater than 90% yield.[16] Reaction of acid **3** with a series



2 **3**

of aldehydes **4** and isocyanides **5** in the presence of Rink resin **6**[17] afforded the four-component Ugi products[18] **7** attached to the polymer. After washing the resin, concomitant hydrolysis of the *tert*-butyl ester group and product cleavage from the polymer was accomplished using 10% TFA in CH_2Cl_2 to provide the desired compounds **1a-1r** (Table 1) in greater than 90% purity and in good overall yield as indicated by ^1H NMR. These compounds were synthesized as racemates and were tested without further purification.[19]



3 **7** **1a-1r**

Results and Discussion

Several advantages over the solution phase synthesis of these NH-acyl-α-aminoamides are realized when the Rink resin amine is employed as a reaction component. Not only are reasonably complex

products readily synthesized using the Ugi one-pot reaction but purification is greatly simplified relative to the solution phase approach. The resin serves as a solid support ammonia equivalent in the four-component condensation. This provides an unsubstituted amide in the final product. Although a wide variety of amines participate in the Ugi reaction, when ammonia is employed a significant number of unidentified side products are formed.[20]

The results from screening compounds **1a-1r** against the target enzyme HePTP using *p*-nitrophenyl phosphate (*p*-NPP) as substrate are shown in Table 1.[21] The data indicate that changes in the potency of

Table 1. Inhibition of HePTP by Cinnamic Acid Derivatives

Compound Number	R_1	R_2	IC_{50} (μM)
1a	*n*-hexyl	*n*-butyl	9
1b	*n*-hexyl	*tetra*-butyl	7.5
1c	*n*-hexyl	cyclohexyl	9
1d	*n*-hexyl	benzyl	6.0
1e	*n*-hexyl	CH_2COOH	7.5
1f	*n*-hexyl	CH_2COOMe	7.2
1g	*n*-hexyl	CH_2COOEt	10
1h	phenyl	*n*-butyl	6.7
1i	phenyl	*tert*-butyl	4
1j	phenyl	cyclohexyl	6.2
1k	phenyl	benzyl	3.9
1l	phenyl	CH_2COOH	10.4
1m	phenyl	CH_2COOMe	20.2
1n	phenyl	CH_2COOEt	9.6
1o	methyl	benzyl	7.2
1p	ethyl	benzyl	15
1q	propyl	benzyl	6.1
1r	butyl	benzyl	6.3

inhibition are much more pronounced for variations in R_2 rather than R_1. For instance, when R_2 is benzyl and R_1 varies from methyl (C1) to *n*-hexyl (C6), only a two fold variation in IC_{50} is observed. In contrast, approximately 6-fold differences in IC_{50} values are observed when R_1 is fixed and R_2 is varied. Thus, an obvious improvement in activity (6-fold) is seen for the case when R_1 is fixed as phenyl and R_2 is changed from CH_2COOMe (20 μM) to benzyl (3.9 μM). The most potent compound in this series (entry **1k**, IC_{50} 3.9 μM) has R_1=phenyl and R_2=benzyl. Further exploration of this series of compounds will be published in due course.

In conclusion, this paper describes the synthesis of a complex variety of NH-acyl-α-aminoamides on Rink resin in addition to the discovery of cinnamic acid derivatives and their biological evaluation against HePTP. These small molecule inhibitors have the potential to serve as a core structure for the synthesis of more potent inhibitors of HePTP and other PTPases.

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15. Compound **3** is synthesized as follows: To *p*-bromobenzoic acid (**2**) (2.0 g, 10 mmol) in dry Et_3N (2.7 mL, 20 mmol) was added *tert*-butyl acrylate (1.6 g, 12.5 mmol), $Pd(OAc)_2$ (2.2 mg, 0.1 mmol) and (tri-*o*-tolyl)phosphine (0.12 g, 0.4 mmol) under argon. The resulting mixture was heated at 100°C for 2 h. H_2O (20 mL) was added and the mixture was acidified with 1.0 M citric acid and extracted with EtOAc (3x20 mL). The combined organic phases were filtered through Celite, dried over Na_2SO_4 and evaporated *in vacuo*. Compound **3** was obtained in 90% yield as a light yellow solid. ^1H NMR (400 MHz, $CDCl_3$) δ 1.46 (s,9H), 6.42 (d,J=16Hz,1H), 7.56-7.59 (m,3H), 8.12 (d,2H); electrospray MS (negative ion) *m/z* 247.0 for $[M-H]^-$.

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19. All compounds were characterized by ^1H NMR and mass spectral analysis. A typical procedure: To Fmoc protected Rink resin (0.025 mmol) was added 20% piperidine in DMF (1.0 mL) at 25°C for 10 min. The solvent was filtered and the step was repeated to achieve complete deprotection. The polymer was washed with CH_3CN (3x1.0 mL) to give **6**. Heptaldehyde in CH_2Cl_2 (0.4 mL, 0.5 M) was added and the mixture was agitated at 25°C. After 1 h, acid **3** in MeOH (0.2 mL, 0.5 M) and ethyl isocyanoacetate in MeOH (0.2 mL, 0.5 M) were added and the mixture was agitated for 72 h. The resin was filtered and washed with DMSO and CH_2Cl_2. A solution of 10% TFA in CH_2Cl_2 (500 μL) was added and the mixture was agitated at 25°C. After 20 min, the solution was filtered and the polymer was washed with additional 10% TFA in CH_2Cl_2 (300 μL). The solvent was evaporated *in vacuo* to provide compound **1g** (5.1 mg, 49% yield). ^1H NMR (400 MHz, CD_3OD) δ 0.84 (t,3H),

1.22 (t,3H), 1.30-1.46 (m,8H), 1.73-1.95 (m,2H), 3.90 (q,2H), 4.12 (m,2H), 4.56 (t,1H), 6.56 (d,J=16Hz,1H), 7.64-7.93 (m,5H); electrospray MS (neg ion) *m/z* 417.0 for $[M-H]^-$.

20. The side products may result from the addition of the isocyanide to an intermediate *bis*-imino aminal formed between two equivalents of ammonia and three equivalents of aldehyde, see: Hunter, D. H.; Sim, S. K. *J. Am. Chem. Soc.* **1969**, *91*, 2602-2603.

21. HePTP was expressed in *E-coli* and purified to homogeneity over two columns. The crude, neat compounds were dissolved in 1.25 mL of DMSO to give 10 mM stock solutions. HePTP tolerates up to 20% DMSO in the final assay concentration. The enzyme activity was assayed in 96-well microtiter plates, using 10 μL of enzyme at 2 μg/mL in assay buffer (100 mM sodium acetate pH 6.0, 1 mM EDTA, 0.1% TritonX-100 and 15 mM β-mercaptoethanol), 10 μL of 2 mM *p*-nitrophenylphosphate and 70 μL of assay buffer. 10 μL of inhibitor stock solution was added to yield a final assay volume of 100 μL. The assay was incubated at 37°C for 65 min, 10 μL of 0.5 M NaOH-50% EtOH was added, and activity was determined by reading absorbance at 405 nm. Inhibition is relative to 500 μM sodium pervanadate (100%) and buffer blank with no enzyme (0%). Detailed kinetic studies as well as enzyme isolation and purification protocols will be published elsewhere.

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