Pergamon

Bioorganic & Medicinal Chemistry Letters 9 (1999) 1469–1474

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

METALLOPEPTIDE APPROACH TO THE DESIGN OF BIOLOGICALLY ACTIVE LIGANDS: DESIGN OF SPECIFIC HUMAN NEUTROPHIL ELASTASE INHIBITORS

Yiqun Shi* and Shubh Sharma*

Palatin Technologies Inc., 175 May Street, Suite 500, Edison Corporate Center, Edison, NJ 08837, U.S.A.

Received 11 February 1999; accepted 15 April 1999

Abstract: A series of compounds was designed to inhibit human neutrophil elastase utilizing a modified metallopeptide scheme developed in our laboratory termed *m*etal-ion *i*nduced *d*istinctive *a*rray of *s*tructures (MIDAS). These metallopeptides, synthesized by solution and solid-phase methods, exhibited excellent structural diversity and specificity in inhibiting human neutrophil elastase.

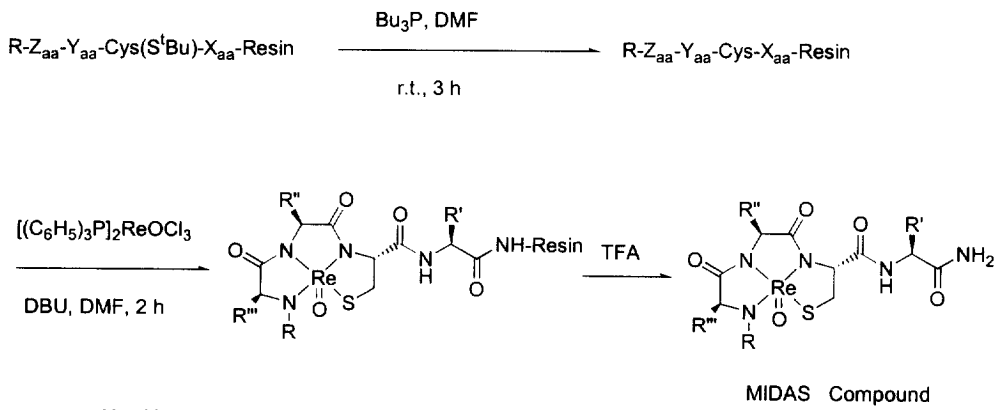
Natural peptides play crucial roles in biological systems. However, their pharmaceutical utilization remains unrealized for various reasons that include their interaction with multiple receptor subtypes, rapid proteolytic degradation, and low bioavailability due to characteristic polar backbone structures that prevent their penetration across cell membrane barriers. To overcome these disadvantages, research efforts have largely focused on developing peptidomimetics in which either a peptide backbone structure is modified[1] or conformationally constrained transition-state analogues are designed.[2] Focus of our current work is the design of target specific compounds on a well defined rigid scaffold obtained after complexing a predesigned amino acid sequence with a rhenium metal ion. The rigid backbone conformation of this metal-peptide scaffold is similar to a reversed-turn structure that is a common structural motif for a variety of peptide hormones and neurotransmitters. The scaffold is then decorated with functional side chain groups to induce specificity and potency for a biological target. Since the amide protons are removed during rhenium ion chelation, the resulting compounds are more closer to fused ring organic molecules rather than the natural peptides. These compounds are air and moisture stable in solid as well as in solution forms over wide acidic and basic conditions.

Both solid- and solution-phase methods have been developed for rapid syntheses of these metallopeptide complexes. First, the predesigned linear peptide sequences were synthesized by the methods of solid-phase peptide synthesis (SPPS). These sequences incorporated a cysteine or cysteine substitute. The sulfhydryl group of the cysteine sidechain constituted the key first step of metal ion complexation during its

0960-894X/99/$ - see front matter
PII: S0960-894X(99)00217-6

reaction with the metal transfer reagent, $ReOCl_3(PPh_3)_2$ (oxotrichlorobis(triphenylphosphine)rhenium[V]).[3] The formation of the desired peptide–metal complex was thermodynamically favored. The solution-phase metal ion complexation protocols consisted of refluxing the mixture of a linear peptide and $ReOCl_3(PPh_3)_2$ in methanol for one half hour in the presence of sodium acetate. HPLC analysis confirmed the completion of the reaction. Solid-phase metal complexation procedures outlined in Scheme 1 were developed to facilitate rapid synthesis of these metallopeptides. This strategy also allowed implementation of a combinatorial library strategy for drug discovery. In brief, after assembling the peptide sequence on appropriate resin using standard SPPS methods, the thio-*tert*-butyl group from cysteine was selectively removed by treatment of peptide resin with tributylphosphine in DMF for 3 h. After washing the resin several times with DMF, 4 equiv of $ReOCl_3(PPh_3)_2$ and 8 equiv of 1,8-diazabicyclic [5.4.0]undec-7-ene (DBU) were added and the mixture was shaken in DMF for 2 hours at room temperature. The final product was obtained after normal SPPS cleavage protocols of TFA cleavage and ether precipitation. The purity level for most of the synthesized metallo-compounds was at least 80% before any HPLC purification.

Scheme 1. Solid-phase syntheses of MIDAS compounds



We have explored this approach to the design and synthesis of metallopeptide compounds, termed *m*etal-ion *i*nduced *d*istinctive *a*rray of *s*tructures (MIDAS), to develop specific inhibitors of human neutrophil elastase (HNE). This enzyme is expressed on the activated neutrophils and is implicated in the degradation of

connective tissue proteins. The HNE has also been reported to play a major role in the disease states such as pulmonary emphysema,[5] cystic fibrosis,[6] rheumatoid arthritis,[7] and atherosclerosis.[8] The inhibition of HNE appears to correct the imbalance between the protease and endogenous inhibitors. Many peptido-mimetic and small molecule inhibitors targeting HNE have been developed.[4] Here, we report on a series of metallopeptide compounds designed to inhibit human neutrophil elastase.

Our search for HNE inhibitor began with a small library containing 60 compounds with five compounds in each pool. The amino acid sequence of each metallopeptide compound was R-Xaa-Yaa-Cys-Val-NH_2, where R was the capping group and Xaa and Yaa randomly selected amino acids. Enzyme inhibition kinetics were studied according to published procedures.[2,9] We observed potent inhibition when the capping group was an aromatic or bulky aliphatic group. Library deconvolution using iterative synthesis and screening approach followed by additional structural explorations suggested that optimal level of activity was obtained when Xaa was isoleucine and Yaa was lysine having a Nε bulky or aromatic group. Table 1 lists selected inhibitors, screened out of 30 compounds synthesized following the deconvolution process.

Table 1. Inhibition constants (K_i) for human neutrophil elastase (HNE) and porcine pancreatic elastase (PPE).

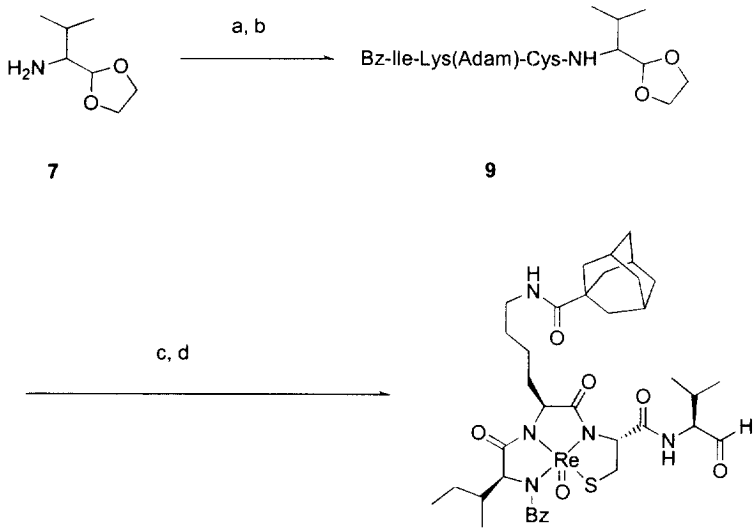
Compound	Structure	K_i (μM)	
		HNE	PPE
1	ReO(v).[**Bz**-Ile-**Lys(Adam)**-Cys-Val-NH_2]	193.7 ± 63.7	
2	ReO(v).[**Ts**-Ile-**Lys(Adam)**-Cys-Val-NH_2]	132.7 ± 48.7	
3	ReO(v).[**Ts**-Ile-**Lys(Adam)**-Cys-Val-**OH**]	36.7 ± 5.6	
4	ReO(v).[**Bz**-Ile-**Lys(Adam)**-Cys-Val-**OH**]	9.7 ± 2.2	4202 ± 1630
5	ReO(v).[**Bz**-Ile-**Lys(Adam)**-Cys-Val-**H**]	5.4 ± 1.2 *	791 ± 280
6	**Bz**-Ile-**Lys(Adam)**-Cys-Val-**OH**	269.7 ± 79.2	

* Slow-binding inhibitor with $k_{on} = 339.3 \pm 6.0\ s^{-1}\ M^{-1}$, $k_{off} = 0.001835 \pm 0.0004\ s^{-1}$

All the compounds, except compound **5**, were fast-equilibrium and reversible inhibitors. The compound **5** was a slow-binding inhibitor and was designed as a putative transition state analog derived from same sequence as those of **1** or **4** with a view to form a tetrahedral covalent adduct with a serine hydroxyl group at the enzyme active site. The K_i value for **5** was much lower than that of **1**, but somewhat closer to that of **4**. The compound **5** with an aldehyde group at C-terminus was synthesized by a solution phase method shown as Scheme 2, where peptide **8** was made by SPPS and compound **7** was synthesized by a reported method.[10] The coupling reaction of **7** with **8**, and reductive deprotection of the thiobutyl group, produced compound **9**. The complexation of **9** with $ReOCl_3(PPh_3)_2$ was conducted in methanol under reflux conditions in the presence of sodium acetate. Final product **5** was obtained after removal of ethylene glycol and

purification on HPLC. Conversion of the C-terminal amide (**1** and **2**) to acid (**3** and **4**) increased the inhibition potency by about 4 to 20-fold. This was in accordance with earlier report suggesting that the carboxylate group might be participating in some sort of specific interactions with the active site of the enzyme.[11]

Scheme 2. The synthesis of compound 5



7 **9** **5**

(a) Bz-Ile-Lys(Adam)-Cys(S^{t}Bu)-OH (**8**), Et_3N, TBTU, DCM. (b) Bu_3P, MeOH.

(c) $[(C_6H_5)_3P]_2ReOCl_3$, NaOAc, MeOH, reflux, 1 h. (d) 1 N HCl, 70 °C, 4 h.

A competition assay was conducted using a known active site specific and irreversible inhibitor, N-methoxysuccinyl-Ala-Ala-Pro-Val-chloromethylketone (ms-AAPV-Cl), and the compound **4**. In this experiment, a mixture of HNE and ms-AAPV-Cl was incubated at 27 °C in the presence of **4**. Aliquots were periodically withdrawn and diluted into a large volume of assay solution for the inhibition assay. If compound **4** reversibly and specifically bound to the active site, it would presumably block the irreversible alkylation of ms-AAPV-Cl. Additionally, dilution would release **4** from the active site of the enzyme so that the remaining enzyme activity would remain higher than that assayed in the absence of compound **4**. As shown in Figure 1, the irreversible inhibition was blocked in the presence of 41.6 μM of **4**. These results, therefore, are consistent with the behavior of competitive, reversible, specific inhibitors. These compounds also showed excellent

selectivity between HNE and porcine pancreatic elastase (PPE). For instance, compound **4** is on the order of 400-fold more potent at inhibiting HNE than PPE (Table 1). This may be due to the valine at P1 position since PPE is reported to have a smaller pocket at the S1 site which is a bit tight in accommodating a valine side chain.[4] The conformation of the rhenium ion-complexed compounds is also markedly different from that of the corresponding linear peptide precursors. For example, linear peptide **6** has very high K_i for HNE compared to rhenium ion-complexed compound **4**. Taken together, these results suggest structural specificity among this series of compounds.

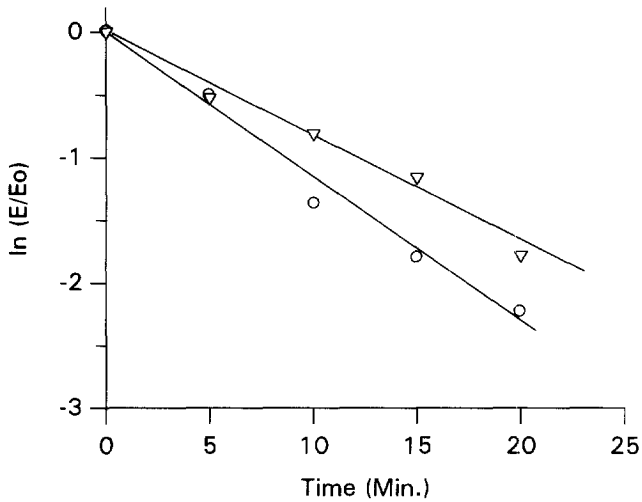


Figure 1. The semi-logarithmic plot of residual enzyme activity as a function of time in a competition experiment of **4** and ms-AAPV-Cl for HNE. [enzyme] = 0.14 unit (Sigma), [ms-AAPV-Cl] = 0.83 μM, incubation temperature is 27°C, and the assay substrate is ms-AAPV-pNA (1 mM). (○) [**4**] = 0 μM, (▽) [**4**] = 41.6 μM

In conclusion, the MIDAS compounds form a new class of compounds with a distinctive global structure derived by the coordination sphere of the complexing metal ion. The syntheses of these compounds are rapid and straight forward. Since a variety of amino acids, natural and unnatural in either L or D optical forms, can be used in constructing the molecular scaffolds, these metallo-compounds present excellent degree of diversity. We have also shown here the development of combinatorial formats of metallopeptides as applied to discovering biological leads. Using a small series of 90 MIDAS compounds we were able to identify specific and reversible binding inhibitors of HNE with low micromolar K_i values. The Re-complexed compounds of this class showed remarkable resistance to proteolysis both *in vitro* and *in vivo* in rodents. This probably is due to participation of amide nitrogens in metal complexation which sterically blocks the

nucleophile from attacking the carbonyl group and disallowing the formation of the sp3 type transition state that is required during amide hydrolysis. The MIDAS compounds also offer a lower hydration and hydrogen bonding potential than the corresponding peptides. It remains to be seen if this feature may make these molecules as potential drug like molecules.

References:

1. (a) Gante, J. *Synthesis* **1989**, 405. (b) Giannis, A.; Kolter, T. *Angew. Chem. Int. Engl.* **1993**, *32*, 1244, and references therein.
2. Stein, R. L.; Strimpler, A. M. *Biochemistry* **1987**, *26*, 2611. (b) Kettner, C. A.; Shenvi, A. B. *J. Biol. Chem.* **1984**, *259*, 15106. (c) Imperiali, B.; Abeles, R. H. *Biochemistry* **1986**, *25*, 3760.
3. Johnson, N.P.; Lock, C.J.L.; Wilkinson, G. *Inorg. Syntheses.* **1967**, *Vol. IX*, 145.
4. Edwards, P.; Bernstein, P. R. *Med. Res. Rev.* **1994**, *14(2)*, 127.
5. *Pulmonary emphysema and proteolysis: 1986*, Taylor, J. C., Mittman, C., Eds, Academic: NY, 1987; pp1-550.
6. (a) Myers, K. C.; Lewandoski, J. R.; Zimmerman, J. J.; Nunley, D.; Calhoun, W. J. and Dopico, G. A. *Am. Rev. Respir. Dis.* **1991**, *144*, 580. (b) Nadel, J. A. *Am. Rev. Respir. Dis.* **1991**, *144*, S48.
7. Janoff, A. In *Neutral Proteases of Human Polymorphonuclear Leukocytes*; Haveerman, K., Janoff, A., Eds.; Urban and Schwarzenberg: Baltimore, 1978; pp 30-417.
8. Janoff, A. Annu. Rev. Med. **1985**, *36*, 207.
9. (a) Segel, I. H. *Enzyme Kinetics*; John Wiley and Sons: New York, 1975. (b) Morrison, J. F.; Walsh, C. T. in *Advances in Enzymology*, Meister, A., Ed.; Wiley: New York, 1988; *Vol. 61*, pp 201-301.
10. Fehrentz, J.; Castro, B. *Synthesis* **1983**, 676.
11. (a) Bourdillon, M. C.; Hornebeck, W.; Soleilhac, J. M.; Robert, L. *Biochem. Soc. Trans.* **1984**, *12*, 876. (b) Hornebeck, W.; Moczar, E.; Szecsi, J.; Robert, L. *Biochem. Pharmacol.* **1985**, *34*, 3315.