Journal of
Medicinal
Chemistry

Volume 41, Number 3 January 29, 1998

Communications to the Editor

Novel Potent Nonpeptide Aminopeptidase N Inhibitors with a Cyclic Imide Skeleton

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Received September 15, 1997

During the metastatic cascade, a tumor cell passes through several connective tissue barriers which contain various adhesive molecules.[1] Proteolytic degradation of the extracellular matrix (ECM) is an important part of the process, and several classes of enzymes have been implicated, including matrix metalloproteinases, serine proteinases, and aminopeptidases.[2−5] Aminopeptidase N (APN) (EC 3.4.11.2/CD13) is a Zn^{2+}-dependent exoprotease that binds to membranes through N-terminal segments as an ecto enzyme. APN is identical to the cell surface antigen CD13, which is expressed not only on myeloid cells but also on many other types of cells.[6−8] Anti-APN/CD13 monoclonal antibody suppresses the process of tumor cell invasion,[9] and a potent inhibitor of APN, bestatin, as well as other inhibitors with different specificities for aminopeptidases, inhibits invasion and matrix degradation by tumor cells in vitro.[5,10,11] It has also been demonstrated that bestatin inhibits experimental and spontaneous metastasis of leukemia[12] and melanoma[13] in mice. These findings suggest that APN plays a crucial role in matrix degradation and invasion by tumor cells and that APN inhibitors may be useful for preventing the spread of malignant cells.

Some of the most intriguing APN inhibitors found to date are natural products such as bestatin[14] and actinonin (Figure 1).[15] These compounds are pseudodipeptides bearing zinc-chelating functionality in the molecule. In general, peptides have drawbacks for clinical application, i.e., low bioavailability, proteolytical lability, rapid biliary excretion, short duration of action, etc. From a medicinal-chemical point of view, it is therefore important to discover nonpeptide derivatives. In this paper, we would like to describe our discovery of *N*-phenyl cyclic imide derivatives as nonpeptide APN inhibitors. Some structure−activity relationships of these novel compounds are also described.

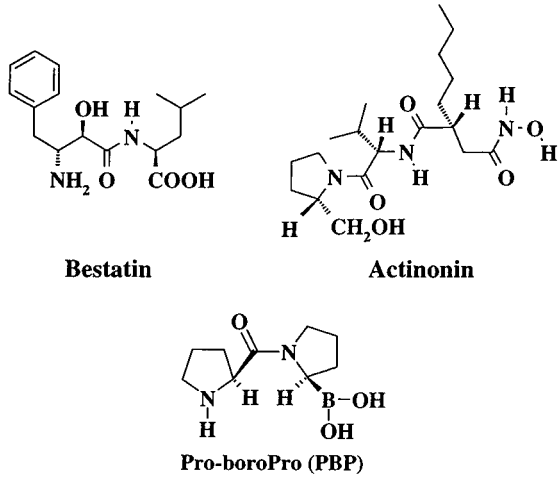

Figure 1. Chemical structures of bestatin, actinonin, and Pro-boroPro (DPP-IV antagonist).

The compounds listed in Table 1 were prepared in good yield by condensation of acid anhydride derivatives and appropriate amine derivatives.[16] The structures and purity were confirmed by elemental analysis and ^{1}H NMR and mass spectroscopy (details will be published elsewhere).

Aminopeptidase N inhibitory activity has been evaluated in intact-cell assays by measuring 7-amino-4-methylcoumarin (AMC) liberated from L-alanine 4-methylcoumaryl-7-amide (Ala-AMC),[9,11] or by measuring *p*-nitroanilide liberated from L-alanine *p*-nitroanilide.[17,18] In our experiments, we adopted the former method, using human acute lymphoblastic leukemia cells, MOLT-4. To confirm the specificity of the inhibition, the compounds were also assayed for inhibition of

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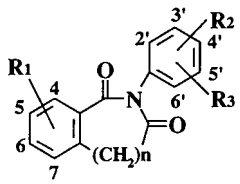
S0022-2623(97)00624-9 CCC: $15.00
Published on Web 01/10/1998

Table 1. APN and DPP-IV Inhibitory Activity of Cyclic Imide Derivatives



compd	*n*	R_1	R_2	R_3	APN[a] IC_{50} (μg/mL)	DPP-IV[b] IC_{50} (μg/mL)
1	0	H	2′-Me	6′-Me	>100	
2	0	H	2′-Et	6′-Et	84.7	>100
3	0	H	2′-iPr	6′-iPr	>100	
4	0	5-NO_2	2′-Et	6′-Et	>100	>100
5	0	5-NO_2	2′-iPr	6′-iPr	>100	
6	0	4-NO_2	2′-iPr	6′-iPr	>100	
7	0	5-NH_2	2′-Me	6′-Me	15.0	23.4
8	0	5-NH_2	2′-iPr	6′-iPr	5.4	81.0
9	0	4-NH_2	2′-Me	6′-Me	>100	>100
10	0	4-NH_2	2′-iPr	6′-iPr	6.7	
11	0	5-OH	2′-Me	6′-Me	10.3	19.8
12	0	5-OH	2′-Et	6′-Et	9.6	12.8
13	0	5-OH	2′-iPr	6′-iPr	15.0	21.3
14	0	4-OH	2′-Me	6′-Me	70.7	
15	0	4-OH	2′-iPr	6′-iPr	4.3	14.1
16	0	fused-Ph[c]	2′-iPr	6′-iPr	>100	
17	1	H	H	H	93.7	>100
18	1	H	2′-Et	H	17.8	>100
19	1	H	2′-iPr	H	54.3	>100
20	1	H	4′-iPr	H	>100	>100
21	1	H	2′-OMe	H	29.5	>100
22	1	H	2′-SMe	H	0.90	>100
23	1	H	2′-Me	6′-Me	41.7	>100
24	1	H	2′-Me	5′-Me	16.0	>100
25	1	H	2′-Me	4′-Me	49.5	>100
26	1	H	2′-Me	3′-Me	30.0	>100
27	1	H	3′-Me	5′-Me	6.2	>100
28	1	H	2′-Et	6′-Et	0.12	>100
29	1	H	2′-iPr	6′-iPr	3.5	>100
30	1	H	2′-Cl	6′-Cl	44.1	>100
actinonin					0.32	>100
bestatin					0.81	>100
PBP					>100	18.3

[a] Aminopeptidase N inhibitory activity was assayed by the L-Ala-MCA method. [b] Peptidyl peptidase inhibitory activity was assayed by the glycyl-L-Pro-MCA method. [c] *N*-(2,6-Diisopropylphenyl)naphthalimide.

dipeptidyl peptidase IV (DPP- IV) (EC 3.4.14.5/CD26), a membrane-associated ecto enzyme (serine protease) of certain subsets of leukocytes, particularly CD4+ T cells.[19,20] The enzymatic activity of intact cells was determined with glycyl-L-proline 4-methylcoumaryl-7-amide (Gly-Pro-AMC) as a substrate. The results are summarized in Table 1.

Structure−Activity Relationships of the Novel Series of *N*-Phenylphthalimides. As can be seen from Table 1, *N*-(2,6-diisopropylphenyl)phthalimide (**3**), a potent inhibitor of TNF-α production,[16] did not show APN inhibitory activity (IC_{50} >100 μg/mL). Introduction of an electron-withdrawing nitro group at the fused benzene ring did not affect the activity. On the other hand, the compounds that have an electron-donating group, such as amino (**7**−**10**) or hydroxyl (**11**−**15**), showed moderate to potent APN inhibitory activity, except compound **9**. The importance of the steric effect of the substituent at the imide nitrogen part of the molecule is clear. In a series of active succinimide derivatives, the activity increased in the order of 2,6-dimethylphenyl < 2,6-diisopropylphenyl (although **11** and **13** exhibited almost the same APN inhibitory activity). Although some of the *N*-phenylphthalimide analogues showed moderate APN inhibitory activities, their specificity was low; i.e., they also showed DPP-IV inhibitory activities with IC_{50} values similar to those for the APN inhibitory activities (Table 1).

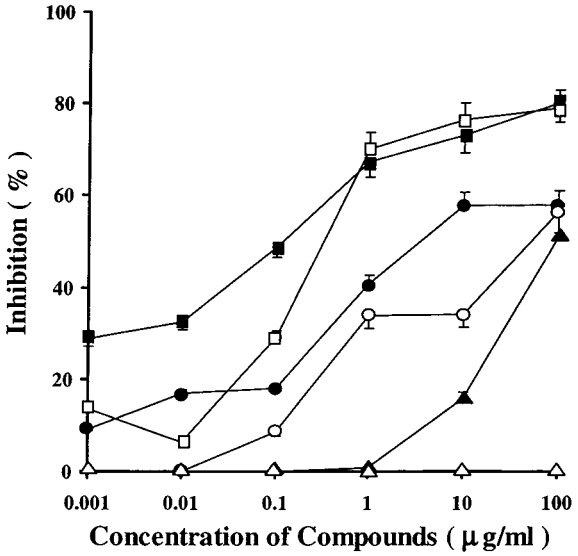


Figure 2. Dose−response curves of the typical homophthalimides and actinonin for inhibition of APN activity: (□) actinonin; (△) **2**; (▲) **17**; (○) **23**; (■) **28**; (●) **29**.

Structure−Activity Relationships of the Novel Series of *N*-Phenylhomophthalimides. When the cyclic imide part was changed from a five-membered ring system to a six-membered one, an extraordinary enhancement of APN inhibitory activity was obtained. *N*-(2,6-Dimethylphenyl)phthalimide (**1**) lacked APN inhibitory activity (IC_{50} > 100 mg/mL), but its homophthalimide derivative (**23**) did show APN inhibitory activity. Nonsubstituted *N*-phenylhomophthalimide (**17**) was almost inactive, but the introduction of a 2-methylthio group (**22**) resulted in very potent inhibition. The steric effect of substituents introduced at the imide nitrogen of the homophthalimides is also important for potent activity. In a series of *N*-(2,6-disubstituted phenyl)homophthalimides, the activity increased in the order of unsubstituted phenyl < 2,6-dimethylphenyl < 2,6-diisopropylphenyl. This rank order is the same as in *N*-(2,6-disubstituted phenyl)phthalimides. But, to our surprise, the 2,6-diethylphenyl derivative (**28**) showed extremely potent APN inhibitory activity, being more potent than actinonin, although the steric bulk of the 2,6-diethylphenyl group is less than that of the 2,6-diisopropylphenyl group (dose−response curves of typical compounds are shown in Figure 2). A similar tendency was also observed for monosubstituted *N*-phenylhomophthalimides (**17**−**19**) [and disubstituted *N*-phenylphthalimide (**2**), though the data are not clear]. This might means that there is a specific steric requirement for tight binding of these types of compounds to APN, although the structure−activity relationships of the regioisomers of the dimethylated analogues (**23**−**27**) are unclear. Further analysis of the structure−activity relationships is in progress.

It should be noted that these *N*-phenylhomophthal-

imides seem to be specific inhibitors of APN, because they are inactive toward another protease DPP-IV (Table 1).

In conclusion, novel and specific non-peptide APN inhibitors were found, with some being much more potent than the naturally occurring APN inhibitors bestatin and actinonin. These compounds (especially **22** and **28**) seem to be specific to APN and should be superior lead compounds for the development of low-molecular-weight non-peptide APN inhibitors for invasion-preventing therapy. Structural optimization and further pharmacological evaluation are in progress.

Acknowledgment. This work was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

Supporting Information Available: Experimental details for compounds listed in Table 1 (5 pages). Ordering information is given on any current masthead page.

References

(1) Fidler, I. J.; Gersten, D. M.; Hart, I. R. The biology of cancer invasion and metastases. *Adv. Cancer. Res.* **1978**, *28*, 149−250.
(2) Liotta, L. A.; Tryggvason, K.; Garbisa, S.; Hart, I.; Foltz, C. M.; Shafie, S. Metastatic potential correlates with enzymatic degradation of basement-membrane collagen. *Nature* **1980**, *284*, 67−68.
(3) Liotta, L. A.; Goldfarb, R. H.; Brundage, R.; Siegel, G. P.; Teranova, V.; Garbisa, S. Effect of plasminogen activator (urokinase), plasmin, and thrombin on glycoprotein and collagenous components of basement membrane. *Cancer Res.* **1981**, *41*, 4629−4636.
(4) Sloane, B. F.; Honn, K. V.; Sadler, J. G. Cathepsin B activity in B16 melanoma cells: a possible marker for metastatic potential. *Cancer Res.* **1982**, *42*, 980−986.
(5) Saiki, I.; Murata, J.; Watanabe, K.; Fujii, H.; Abe, F.; Azuma, I. Inhibition of tumor-cell invasion by ubenimex (bestatin) *in vitro*. *Jpn. J. Cancer Res.* **1989**, *80*, 873−878.
(6) Look, A. T.; Ashmun, R. A.; Shapiro, L. H.; Peiper,S. C. Human myeloid plasma membrane glycoprotein CD13 (gp 150) is identical to aminopeptidase N. *J. Clin. Invest.* **1989**, *83*, 1299−1307.
(7) Bowes, M. A.; Kenny, A. J. An immunohistorical study of endopeptidase-24.11 and aminopeptidase N in lymphoid tissues. *J. Immunol.* **1987**, *60*, 247−253.
(8) Nagaoka, I.; Yamashita,T. Leucine aminopeptidase as an ectoenzyme of polymorphonuclear neutrophils. *Biochim. Biophys. Acta* **1987**, *598*, 169−172.
(9) Saiki, I.; Fujii, H.; Yoneda, J.; Abe, F.; Nakajima, M.; Tsuruo, T.; Azuma, I. Role of aminopeptidase N (CD13) in tumor-cell invasion and extracellular matrix degradation. *Int. J. Cancer.* **1993**, *54*, 137−143.
(10) Yoneda, J.; Saiki, I.; Fujii, H.; Abe, F.; Kojima, Y.; Azuma, I. Inhibition of tumor invasion and extracellular matrix degradation by ubenimex (bestatin). *Clin. Exp. Metastasis* **1992**, *10*, 49−59.
(11) Fujii, H.; Nakajima, M.; Aoyagi, T.; Tsuruo, T. Inhibition of tumor cell invasion and matrix degradation by aminopeptidase inhibitors. *Biol. Pharm. Bull.* **1996**, *19*, 6−10.
(12) Tsuruo, T.; Naganuma, K.; Iida, H.; Yamori, T.; Tsukagoshi, S.; Sakurai, Y. Inhibition of lymph node metastasis of P388 leukemia by bestatin in mice. *J. Antibiot.* **1981**, *34*, 1206−1209.
(13) Maroux, S.; Louvard, D.; Baratti, B. The aminopeptidase from hog intestinal brush border. *J. Biochim. Biophys. Acta* **1973**, *321*, 282−295.
(14) Umezawa, H.; Aoyagi, T.; Suda, H.; Hamada, M.; Takeuchi, T. Bestatin, an inhibitor of aminopeptidase B, produced by actinomycetes. *J. Antibiot.* **1976**, *29*, 97−99.
(15) Umezawa, H.; Aoyagi, T.; Tanaka, T.; Okuyama, A.; Nagasawa, H.; Hamada, M.; Takeuchi, T. Production of actinonin, an inhibitor of aminopeptidase M, by actinomycetes. *J. Antibiot.* **1985**, *38*, 1629−1630.
(16) (a) Sasaki, K.; Shibata, Y.; Hashimoto, Y.; Iwasaki, S. Benzylphthalimides and phenethylphthalimides with thalidomide-like activity on the production of tumor necrosis factor alpha. *Biol. Pharm. Bull.* **1995**, *18*, 1228−1233. (b) Shibata, Y.; Sasaki, K.; Hashimoto, Y.; Iwasaki, S. Phenylphthalimides with tumor necrosis factor alpha production-enhancing activity. *Chem. Pharm. Bull.* **1996,** *44*, 156−162. (c) Miyachi, H.; Azuma, A.; Hioki, E.; Iwasaki, S.; Hashimoto, Y. Enantio-dependence of inducer-specific bidirectional regulation of tumor necrosis factor (TNF)-alpha production: Potent TNF-alpha production inhibitors. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2293−2298.
(17) Bauvois, B.; Sanceau, J.; Wietzerbin, J. Human U937 cell surface peptidase activities: characterization and degradative effect on tumor necrosis factor alpha. *Eur. J. Immunol.* **1992**, *22*, 923−930.
(18) Laouar, A.; Wietzerbin, J.; Baubois, B. Divergent regulation of cell surface protease expression in HL-60 cells differentiated into macrophages with granulocyte macrophage colony stimulating factor or neutrophins with retinoic acid. *Int. Immunol.* **1993**, *5*, 965−973.
(19) Schon, E.; Jahn, S.; Kiessig, S. T.; Demuth, H. U.; Neubert, K.; Barth, A.; Baehr, R.; Ansorge, S. The role of dipeptidyl peptidase IV in human T lymphocyte activation. Inhibitors and antibodies against dipeptidyl peptidase IV suppress lymphocyte proliferation and immunoglobulin synthesis *in vitro*. *Eur. J. Immunol.* **1987**, *17*, 1821−1826.
(20) Flenke, G. R.; Munoz, E.; Huber, B. T.; Plaut, A. G.; Kettner, C. A.; Bachovchin, W. W. Inhibition of dipeptidyl peptidase IV (DP-IV) by Xaa-boroPro dipeptides and use of these inhibitors to examine the role of DP-IV in T-cell function. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 1556−1559.

JM970624O