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Research paper

Novel leucine ureido derivatives as aminopeptidase N inhibitors. Design, synthesis and activity evaluation

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ABSTRACT

Aminopeptidase N (APN/CD13) over-expressed on tumor cells and tumor microenvironment, plays critical roles in tumor invasion, metastasis and angiogenesis. Here we described the design, synthesis and preliminary activity studies of novel leucine ureido derivatives as aminopeptidase N (APN/CD13) inhibitors. The results showed that compound **7a** had the most potent inhibitory activity against APN with the $\mathbf{IC_{50}}$ value of 20 nM, which could be used for further anticancer agent research.

1. Introduction

Aminopeptidase N (APN, also known as CD13; EC 3.4.11.2) is a zinc-dependent type II membrane-bond ectopeptidase that preferentially releases neutral and basic amino acids from the N-terminus of oligopeptides. It has been identified as a cell surface marker for malignant myeloid cells [1] and was over-expressed on various mammalian tumor cells [2], such as melanoma, prostate, ovarian, colon, renal and pancreas carcinomas, as well as tumor microenvironment [3]. APN degrades extracellular matrix (ECM) which is the main barrier of malignant cell dissemination to promote tumor invasion and metastasis [4]. More and more researches indicate that APN is an important angiogenic factor and can serve as a target for delivering drugs into tumors and inhibiting angiogenesis [3,5]. Moreover, APN is a mark and therapeutic target in human liver cancer stem cells which are responsible for tumor resistance to chemo/radiation therapy as well as tumor relapse and progression [6–8]. Accordingly, APN is considered as an important target for anti-tumor research. Therefore, the design and synthesis of APN inhibitors may have clinical significance for the discovery of anticancer agents.

To date, many natural or synthetic small APN inhibitors have been reported. Natural APN inhibitors include Bestatin [9], Probestin [10], AHPA-Val [11], Lapstatin [12], Amastatin [13], *etc.* Synthetic small APN inhibitors include α-aminoaldehydes [14], α-aminophosphiric acids [15], α-aminoboronic acids [16], L-lysine derivatives [17], L-arginine derivatives [18], cyclic-imide derivatives [19,20], β-dicarbonyl derivatives [21] and so on. However, Bestatin is the only marketed APN inhibitor. Low inhibitory activity is the obstruction of APN inhibitors development. So it is necessary to exploit more active APN inhibitors by rational drug design.

In our previous work, we have reported a series of ureido derivatives [22]. The biological characterization revealed that some compounds displayed potential inhibitory activity against APN. Especially, the IC_{50} value of compound **4k** was 2.7 μM compared with 9.1 μM of Bestatin. Herein, in order to find better APN inhibitors, Compound **4k** was used as the lead compound. In our series of L-arginine derivatives, we found that compounds containing disubstituted groups on phenyl had better activities than those containing unsubstituted or single substitution [18]. The same tendency exists in our L-lysine derivatives [17]. Based on these observations, a series of novel leucine ureido derivatives (**7a**–**7k**) containing different disubstitution on the phenyl group of **4k** have been synthesized, meanwhile, some analogues (**8a**–**8g**) with disubstituted benzyl groups replaced the phenyl group of **4k** have also been obtained (Fig. 1). The *in vitro* inhibitory activity was

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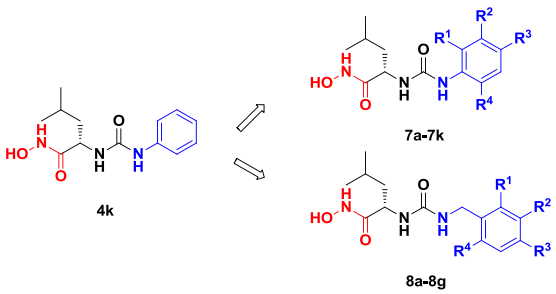


Fig. 1. The new APNIs derived from compound **4k** (two of R are H).

measured against APN enzyme. Additionally, the antimetastasis effect of potent compounds was evaluated *in vitro* and *in vivo* assay.

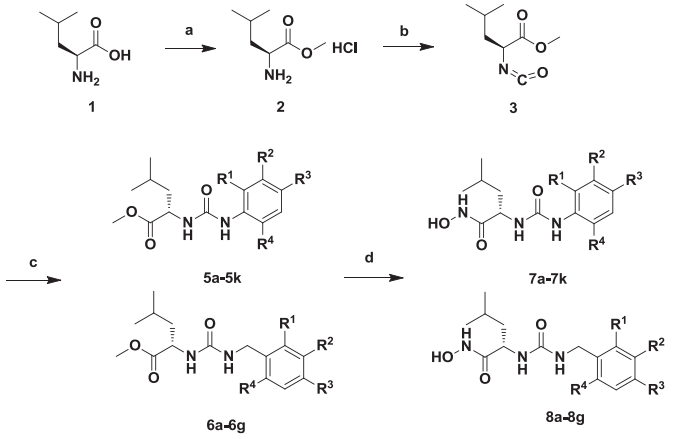
2. Chemistry

The target compounds were synthesized efficiently via the route outlined in Scheme 1. Starting from commercially available L-leucine, the key intermediate leucine isocyanate was obtained via esterification and isocyanate, and then coupled with the corresponding disubstituted anilines or benzylamines. Without further purification, they were directly transformed into hydroxamic acids as the target products.

3. Result and discussion

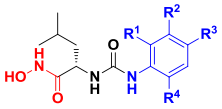
The target compounds were evaluated for their inhibitory activities toward **APN** and **HDACs** by the spectrophotometric method as described previously [23,24]. Similar to **APN**, **HDACs** are zinc-dependent metalloproteinases as well and associated closely with the invasion and metastasis of tumor [25,26]. Thereby the assay was performed on both **APN** and **HDACs** so as to identify the selectivity of our target compounds, all the inhibition results are summarized in Tables 1 and 2. Bestatin was used as the positive control for **APN** inhibitor, while, SAHA was used as the positive control for **HDACs** inhibitor.

As shown in Tables 1 and 2, it is worthy to note that these ureido derivatives displayed dramatic selectivity towards **APN** over **HDACs**. These results, to a certain extent, validated our strategy for design of potential APNIs. As the above mentioned selectivity



Scheme 1. Reagents and conditions: (a) acetyl chloride, MeOH, reflux; (b) triphosgene, $NaHCO_3$, DCM, ice-bath; (c) corresponding disubstituted anilines or benzylamines, DCM, room temperature; (d) NH_2OK, MeOH, 1 h.

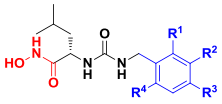
Table 1
Structures and IC_{50} of 7a–7k against APN and HDACs.



Compd	R^1	R^2	R^3	R^4	$IC_{50}(\mu M)^a$ APN	$IC_{50}(\mu M)^a$ HDAC
7a	Me	H	H	Me	0.02 ± 0.01	>100
7b	Et	H	H	Et	0.03 ± 0.02	>100
7c	iPr	H	H	iPr	0.05 ± 0.03	>100
7d	F	H	H	F	0.23 ± 0.14	>100
7e	Me	H	Me	Me	0.08 ± 0.02	>100
7f	Me	H	Me	H	0.18 ± 0.13	>100
7g	F	H	F	H	0.34 ± 0.19	>100
7h	Cl	H	Cl	H	0.56 ± 0.23	>100
7i	OMe	H	OMe	H	0.46 ± 0.23	>100
7j	H	F	OMe	H	0.42 ± 0.12	>100
7k	H	Cl	Me	H	0.09 ± 0.05	>100
4k	H	H	H	H	1.15 ± 0.53	>100
Bestatin	–	–	–	–	3.40 ± 0.03	>100
SAHA	–	–	–	–	–	0.12 ± 0.03

[a] Mean values and standard deviations of triplicate experiments are given.

Table 2
Structures and IC_{50} of 8a–8g against APN and HDACs.



Compd	R^1	R^2	R^3	R^4	$IC_{50}(\mu M)^a$ APN	$IC_{50}(\mu M)^a$ HDAC
8a	F	H	H	F	0.69 ± 0.25	>100
8b	Cl	H	H	Cl	0.58 ± 0.14	>100
8c	F	H	F	H	0.33 ± 0.21	>100
8d	OMe	H	OMe	H	0.46 ± 0.19	>100
8e	H	F	F	H	0.42 ± 0.17	>100
8f	H	Me	Me	H	0.44 ± 0.16	>100
8g	Me	Me	H	H	0.07 ± 0.02	>100
4k	–	–	–	–	1.15 ± 0.53	>100
Bestatin	–	–	–	–	3.40 ± 0.03	>100
SAHA	–	–	–	–	–	0.12 ± 0.03

[a] Mean values and standard deviations of triplicate experiments are given.

against APN, the following SARs were mainly discussed about APN inhibition.

Bioassay results indicate that all compounds exhibited better inhibitory activity against **APN** than the leading compound **4k**, some of which had 10-fold or more improvement. It may be due to the disubstitued groups on the phenyl group can enhance the interaction with the hydrophobic region of the enzyme. The activity of analogues with disubstituted anilines (**7a**–**7k**) is better than analogues with disubstituted benzylamines (**8a**–**8g**). Comparing **7a**–**7d** and **7f**–**7k**, we could find that the compounds containing ortho disubstituted phenyl groups have better activities than those containing other disubstituted phenyl groups. Among compounds **7a**–**7d** with ortho disubstitution, **7a**–**7c** with dialkyl groups on the benzene ring were more potent than **7d** with dihalogens. And different alkyl groups in aromatic ring also have different levels of impacts on their activities. The data shown in Table 1 suggested that the preferred substitutions against APN were, in decreasing order, methyl-substitution (**7a**) and ethyl-substitution (**7b**), followed by the isopropyl inhibitor (**7c**). It seems that methyl is the most favorable group for APN and bigger bulks lead to impaired activity. Compound with tri-substitution (**7e**) on the aromatic ring