Bioorganic Chemistry 81 (2018) 672–680

Contents lists available at ScienceDirect

Bioorganic Chemistry

journal homepage: www.elsevier.com/locate/bioorg

Discovery of novel cathepsin inhibitors with potent anti-metastatic effects in breast cancer cells

Lei Yuan[a], Lei Sheng[a], Wenhui He[b], Chunyang Zou[c], Baichun Hu[a], Jun Liu[a], Wentao Ge[a], Yang Liu[a], Jian Wang[a,*], Enlong Ma[b,*]

[a] Key Laboratory of Structure-Based Drug Design and Discovery (Shenyang Pharmaceutical University), Ministry of Education, Shenyang 110016, PR China
[b] Department of Pharmacology, Shenyang Pharmaceutical University, Shenyang 110016, PR China
[c] Department of Pharmacy, Liaoning Vocational College of Medicine, Shenyang 110101, PR China

ARTICLE INFO

Keywords:
Cathepsin inhibitor
Antimetastatic effect
Docking calculations
Natural product consensus pharmacophore strategy
Asperphenamate

ABSTRACT

It is still challenging to determine the potential targets of natural products, which is essential for further drug research and development. Due to its novel mechanism of action of inducing autophagy effects in breast cancer cells, asperphenamate has received our considerable attention. However, its unknown target inevitably impedes further study. In our previous work, the target enzyme of asperphenamate was predicted as cathepsin by the natural product consensus pharmacophore strategy. Then, asperphenamate and its three derivatives were chosen to study in detail by molecular docking calculations with AutoDock 4 suite. The docking results showed the three derivatives interacted more tightly with either cathepsin L or cathepsin S than with asperphenamate. The *ortho*-benzyloxyl phenylacetyl derivative **1** and *p*-toluenesulfonyl derivative **3** showed similar interactions with cathepsin L and adopted a better geometric shape within the binding pocket than did the *N*-CBZ-piperidyl analog **2**. On the other hand, compound **2** formed more hydrogen bonds than **1** and **3** to make it tightly bind within cathepsin S. The cathepsin inhibitory activity assay verified the molecular simulation results. Compound **2** was remarkably less active than **1** and **3** against cathepsin L. However, compound **2** showed the strongest potency against cathepsin S with IC_{50} of 13.12 ± 0.29 μM. Considering that cathepsin S plays a vital role in the process of metastasis in breast cancer cells, the inhibitory effect of **2** on migration and invasion was further studied in human breast cancer MDA-MB-231 cells by wound healing and transwell chamber assays. The results illustrated that **2** exhibited an apparent inhibitory ability to the metastasis of MDA-MB-231 cells. Next, **2** will be chosen as a lead compound to develop novel double functional chemotherapeutic agents with both novel mechanisms of action against apoptosis-resistant cancer cells, such as inducing autophagy and inhibiting cancer metastasis.

1. Introduction

Natural products are always considered as a valuable source of bioactive molecules for the development of therapeutic drugs [1–4]. Their exceptionally diverse scaffolds also contribute to finding novel biological mechanisms of action, which may bring breakthroughs to clinical treatment for numerous diseases. Taxol and camptothecin are typical examples that have been approved as the first-line regimen for the treatment of ovarian, breast, lung and colon cancer [5–6]. At present, cancer is still a major threat to human health [7]. Chemotherapy is an effective method in managing patients diagnosed with any form of cancer, but the emergence of chemoresistance affects its efficacy. Accordingly, the need for the development of new drugs with novel mechanism of action is urgent [8].

Asperphenamate, a dipeptide analog, has an *N*, *N*'-substituted phenylalanine-phenylalaninol ester framework and was isolated by our group from raw malt, a traditional medicine for the treatment of hyperplasia of mammary glands [9–10]. It attracted our attention that asperphenamate can inhibit cancer cell proliferation by fully inducing autophagy [11]. However, the unknown action target inevitably impedes further research of asperphenamate. Based on the natural product consensus pharmacophore strategy [12], patriscabratine, a natural analog of asperphenamate, exhibited similar pharmacophore to aurantiamide acetate (Fig. 1) as a natural cathepsin L inhibitor [13]. This result suggested that the target enzyme of patriscabratine should be cathepsin L. Considering the similar pharmacophore skeleton to patriscabratine, it is hypothesized that asperphenamate likely targets the cathepsin enzyme.

* Corresponding authors.
E-mail addresses: jianwang@syphu.edu.cn (J. Wang), Enlong_Ma20180718@126.com (E. Ma).

https://doi.org/10.1016/j.bioorg.2018.09.029
Received 31 July 2018; Received in revised form 10 September 2018; Accepted 18 September 2018
Available online 20 September 2018
0045-2068/

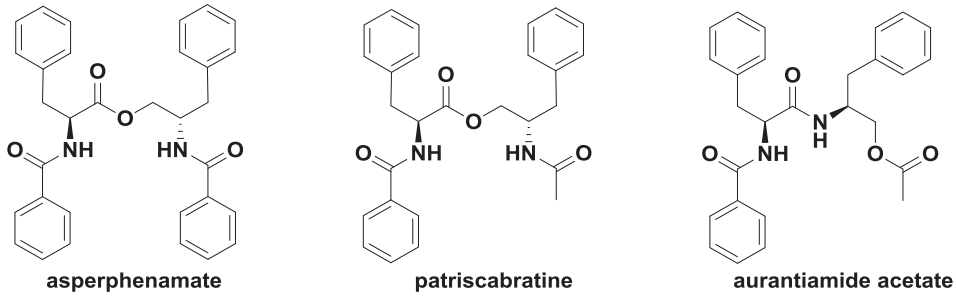

Fig. 1. The structures of asperphenamate and its natural analogs.

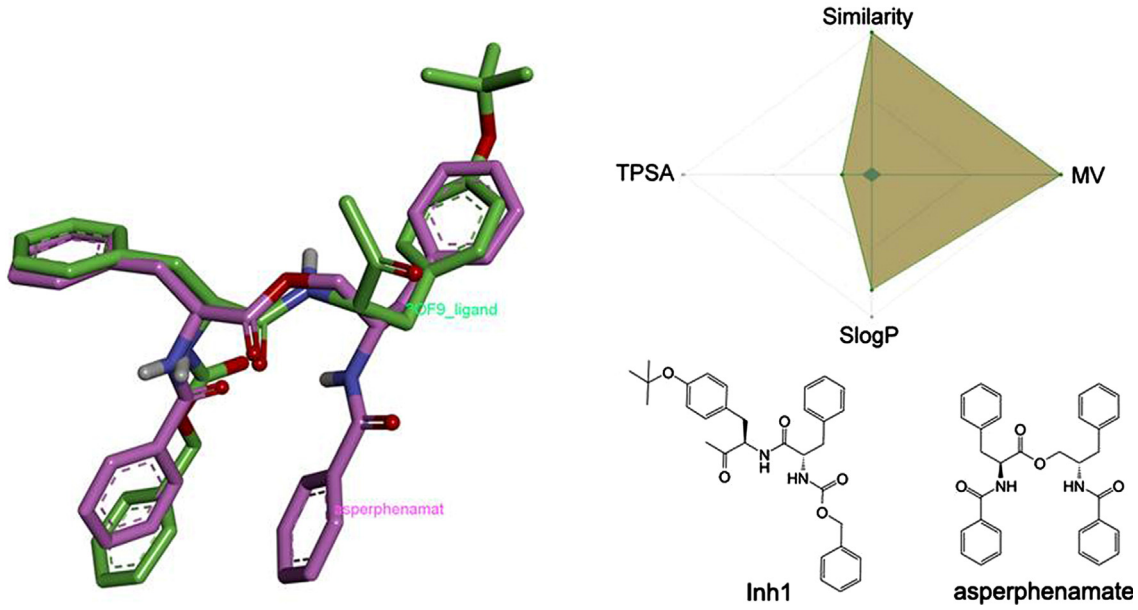

Fig. 2. Molecular similarity analysis of asperphenamate and cocrystal ligand from cathepsin L crystal structure (PDB code 3OF8).

Table 1
Inhibitory activities of asperphenamate and its three derivatives against cathepsins L, S, K and B.

Enzyme	IC_{50} (μM)[a]			
	Cathepsin L	Cathepsin S	Cathepsin K	Cathepsin B
1	46.77 ± 4.46	85.32 ± 1.62	NA[b]	NA
2	88.56 ± 4.77	13.12 ± 0.29	NA	NA
3	30.2 ± 1.12	79.68 ± 1.13	NA	NA
Asperphenamate	91.23 ± 1.26	171.11 ± 1.76	NA	NA

[a] Data are shown as the mean ± SD from three independent experiments.
[b] NA means no activity.

To test this hypothesis, the molecular similarity between asperphenamate and a cocrystal ligand of 3OF8 (cathepsin L) [14], **Inh1**, was analyzed by Forge software [15]. Additionally, the similarity index was calculated based on topology polar surface area (TPSA), similarity, slogP and molecular weight (MW). The results showed that the two compounds have a high similarity in molecular weight, similarity and slogP (Fig. 2). Aiming to validate the calculation results, the inhibitory activity against cathepsins L, S, K and B, which are closely related to tumorigenesis, was evaluated. In accordance with molecular simulation results, asperphenamate showed inhibition effects against cathepsin L. At the same time, it also displayed weak inhibitory ability against cathepsin S (Table 1).

During the past years, numerous asperphenamate derivatives were designed and synthesized by our group because of their ability to induce autophagy in cancer cells. Among these derivatives, three reported

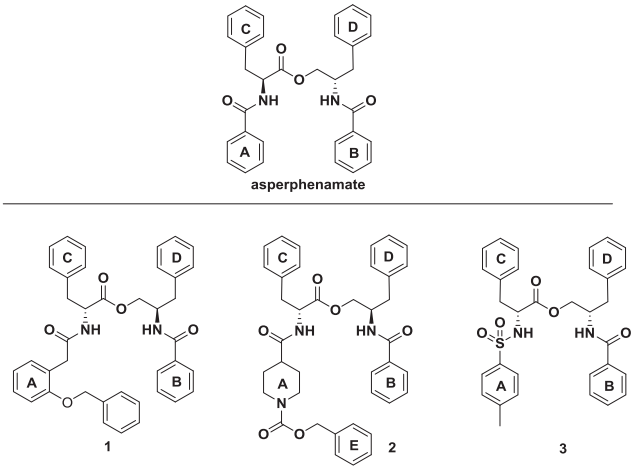

Fig. 3. The structures of synthetic derivatives of asperphenamate.

compounds, namely, *ortho*-benzyloxyl phenylacetyl **1**, *N*-CBZ-piperidyl **2** and sulfonyl derivative **3** (Fig. 3) displayed more potent antiproliferative ability than asperphenamate [11,16–17]. We hypothesized that these compounds may show greater inhibitory effect against cathepsin than asperphenamate does. In the meantime, some studies proved that cathepsins, such as L, B, K, H, S, and X, exhibited increased activity in cancer cells and were associated with the migration and invasion of malignant cells [18–20].

Therefore, molecular docking and cathepsin inhibition activity