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组织蛋白酶 B 的新型天然抑制剂*

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摘要: 组织蛋白酶 B 是木瓜蛋白酶类半胱氨酸蛋白酶家族的重要成员, 它与人类多种疾病相关, 尤其是在恶性肿瘤的侵袭转移过程中扮演了重要角色。通过随机筛选, 发现了五个对组织蛋白酶 B 具有较好抑制活性的天然化合物 prodelphinidin B-2 3′-O-gallate (**1**), prodelphinidin B-2 (**2**), procyanidin B-2 (**3**), puerin A (**4**) 和 (-) epigallocatechin-3-O-gallate (**5**), 其 IC_{50}值分别为 0.58, 0.44, 0.76, 2.07 和 0.96 μmol/L。这五个抑制剂为黄烷醇类化合物, 均为组织蛋白酶 B 的新型天然抑制剂。

关键词: 组织蛋白酶 B; 天然抑制剂; 黄烷醇
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New Natural Inhibitors of Human Cathepsin B*

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Abstract: Cathepsin B is an important member of the papain-like cysteine proteases. It has been implicated in the pathology of many human diseases, especially in the invading and metastasis of most malignant tumors. With random screening, five natural compounds, prodelphinidin B-2 3′-O-gallate (**1**), prodelphinidin B-2 (**2**), procyanidin B-2 (**3**), puerin A (**4**) and (-) epigallocatechin-3-O-gallate (**5**), were found to show potent cathepsin B inhibitory activity with IC_{50}s of 0.58, 0.44, 0.76, 2.07 and 0.96 μmol/L, respectively. Inhibitors **1**–**5** are found with chemotype of flavanols, which are new natural inhibitors of cathepsin B.

Key words: Cathepsin B; Natural inhibitors; Flavanols

Cathepsins, the main member of the papain-like cysteine proteases, comprise a group of proteolytic enzymes involved in many human physiological processes, and the overexpression of these enzymes will lead to severe diseases (Lecaille *et al.*, 2002; Turk *et al.*, 2000).

Cathepsin B (CatB) is one of the most extensively investigated members of the papain-like cysteine proteases. It has been involved in various human diseases, such as tumor, osteoporosis, rheumatoid arthritis and parasitic diseases etc (Lecaille *et al.*, 2002; Otto and Schirmeister, 1997; Zeng *et al.*, 2005). Recently, the important role of CatB in tumor metastasis has been well documented, which demonstrated that CatB can enhance the invasion of many tumors by aiding in extracellular matrix breakdown. Moreover, increased expression and/or activity of CatB have been observed in many metastatic tumors. It suggested that CatB is implicated in the development, invasion and metastasis of tumors. Therefore, CatB is considered as an important target in

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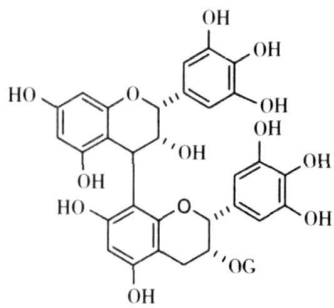
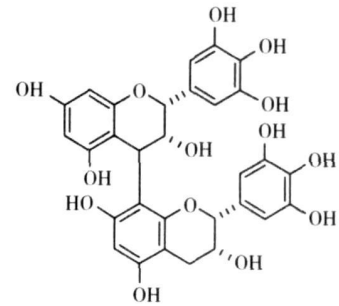
cancer intervention, and its inhibitors are highly sought as potential anticancer and antimetastatic agents (Lecaille *et al.*, 2002; Turk *et al.*, 2000; Zeng *et al.*, 2005; Lim *et al.*, 2004; Koblinski *et al.*, 2000; Berdowska, 2004).

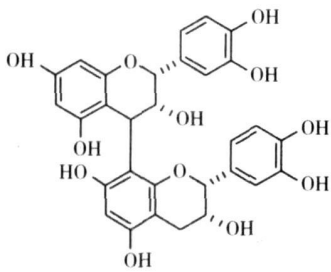
Many anticancer drugs have originated from natural products (Tan *et al.*, 2006). In our previous work, six biflavones isolated from gymnosperm plant and one carbazole alkaloid isolated from *Murraya koenigii* were found to be novel natural inhibitors of human cathepsin B (Zhang *et al.*, 2005; Zeng *et al.*, 2006; Wang *et al.*, 2003). The structure-activity relationship between these six biflavones and CatB was further analyzed (Zeng *et al.*, 2006; Pan *et al.*, 2005). The following screening results of CatB assay indicated that five natural flavanols (**1**– **5**), isolated from various plant resources, were found to show potent inhibitory activity against human cathepsin B (Fig. 1).



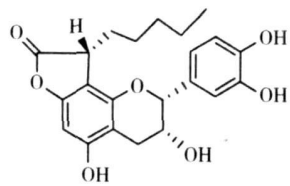
1 (prodelphinidin B-2 3′-O-gallate)



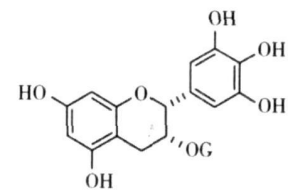
2 (prodelphinidin B-2)



3 (procyanidin B-2)



4 (puerin A)



5 (-) epigallocatechin-3-O-gallate

Fig. 1 Chemical structures of compounds **1**– **5**

Materials and Methods

Inhibitors **1**– **5** were isolated from plants (Table 1), and detailed purification and identification of these compounds were described by us previously (Zhou and Yang, 2000; Zhou *et al.*, 2005; Zhou *et al.*, 2000). Purities of these compounds were > 95%. Inhibitory activities against human cathepsin B (Cat# 219364, Calbiochem) were determined spectrofluorometrically according to the method we used before (Zeng *et al.*, 2006). Leupeptin (Cat# L9783, Sigma), a well known inhibitor for cathepsins, was used as reference compound in this assay. The IC_{50} values were calculated by dose-response curves with the highest tested concentration of 2.5 μg/mL.

Results and Discussion

Results from CatB inhibition assay showed that compound **1**– **3**, three biflavanols, showed potent CatB inhibitory activities with IC_{50} values of 0.58, 0.44 and 0.76 μmol/L, respectively (Table 1), which indicated that the number of hydroxyl substitution may be has effect on the activity of this kind of inhibitors. Interestingly,

Table 1 Inhibitory activity of compounds **1**– **5** against human cathepsin B

Comp.	Sources	IC_{50} (μmol/L)
1	*Myrica nana*	0.58±0.21
2	*M. nana*	0.44±0.15
3	*Camellia saluenensis*	0.76±0.16
4	*C. sinensis* var. *assamica*	2.07±0.05
5	*Myrica nana*	0.96±0.09
Leupeptin	——	0.05±0.01

most of the natural CatB inhibitors we have found are with dimeric structures, such as alkaloid (Wang *et al.*, 2003), biflavone (Pan *et al.*, 2005; Zeng *et al.*, 2006) and biflavanol (**1–3**).

Tumor cell invasion involves attachment of tumor cells to the underlying basement membrane, local proteolysis and migration of tumor cells through the proteolytically modified region (Koblinski *et al.*, 2000). CatB plays an important role in local proteolysis by degrading extracellular matrix and basement membrane, and enhances tumor invasion and metastasis (Koblinski *et al.*, 2000; Berdowska, 2004; Turk *et al.*, 2000; Zeng *et al.*, 2005). CatB overexpression has been demonstrated in many human tumors including breast, lung, brain, gastrointestinal, head and neck cancer, and melanoma (Koblinski *et al.*, 2000; Berdowska, 2004; Sloane *et al.*, 1981). In vitro and in vivo studies have shown that protease inhibitors can reduce the invasive and metastatic capabilities of tumor cells (Koblinski *et al.*, 2000). Several types of chemical functionality have served as the central pharmacophore for cysteine proteases inhibitors (Lecaille *et al.*, 2002). However, most of inhibitors are peptide-based molecules. In this work, five flavanols were found as novel natural inhibitors of CatB, which provided us the new insight into the research of CatB inhibitors.

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