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Research paper

Design, synthesis and biological evaluation of novel asperphenamate derivatives

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ABSTRACT

A series of novel asperphenamate derivatives were designed and synthesized, including series I (the A-phenyl group replaced with various aromatic heterocycles) and series II (the acyl group substituted by sulfonyl group). All compounds have been screened for their antiproliferative activity *in vitro* against MCF-7, HeLa, and BEL-7402 cell lines by the standard MTT method. Structure–activity relationship studies displayed the heterocycle type played an important role in activity. Six-membered ring derivatives displayed more potency than five-membered ring and the sulfonyl group in A-ring region made an important contribution to activity. Among all derivatives, tosyl derivative **8c** exhibited the greatest potency in three human cancer cell lines. Especially in MCF-7 cells, the cellular potency of **8c** was approximately 3.0-fold more potent than that of cisplatin. Firstly, the mechanism of cell death induced by **8c** in MCF-7 cells was investigated. The results showed that the cell death was induced by autophagy instead of apoptosis or cell cycle arrest. Further studies indicated that **8c** might induce autophagic cell death in HeLa and BEL-7402 cell lines.

1. Introduction

Cancer is one of the most prevalent diseases diagnosed in developed countries and one of the leading causes of mortality [1,2]. The development of cancer therapies over the past few decades has focused principally on chemotherapy combined with other treatment approaches such as surgery, radiotherapy, and targeted therapy. Although a large majority of current anti-cancer drugs exhibit their effects through the induction of apoptosis in cancer cells, many types of cancer are resistant to proapoptotic stimuli. Therefore, new types of drugs that display antiproliferative effects against such apoptosis-resistant cancers are urgently needed.

Autophagy is an important mechanism to maintain cell homeostasis and promote cell survival during starvation [3,4]. But persistent stress or prolonged starvation can result in autophagic cell death, or type II programmed cell death, which is distinct from apoptotic cell death (type I programmed cell death) [5,6]. More recently, induction of autophagy was considered as a mechanism for some anti-cancer compounds and provided a novel target for the development of chemotherapeutic agents [7–11].

Asperphenamate, a dipeptide analog, has an *N, N'*-substituted phenylalanine-phenylalaninol ester framework. It was isolated from raw malt, a traditional medicine for the treatment of mammary hyperplasia [12]. In previous work, we found both the type and position of substituent on A-ring displayed a remarkable influence on bioactivity and *ortho*-benzyloxy substituted derivative (**OBA**) showed the greatest potency. It inhibited the growth of MCF-7 cells by inducing autophagy instead of apoptosis [13]. The results suggested that A-ring region had a significant impact on anticancer effect of asperhenamate.

Heterocyclic compounds occur widely in nature and are essential for life. The widespread use of them as a scaffold in medicinal chemistry establishes this moiety as a member of the privileged structures class [14,15]. Recently, many sulfonyl derivatives have also been reported to display substantial anticancer activity *in vitro*

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and/or *in vivo* [16–19]. In our effort to discover and develop autophagy regulator as potential new anticancer agents, we further investigated the effect of the phenyl and acyl moiety in A-ring region on activity, respectively. And two series of derivatives were designed and synthesized, including series I: the A-phenyl group was replaced by various aromatic heterocycles; series II: the acyl group was substituted by sulfonyl group.

In this paper, we described the synthesis of two series of derivatives as well as their anti-proliferative activities in MCF-7, HeLa, and BEL-7402 cell lines *in vitro*. The cell death mechanisms were further explored in three human cancer cell lines.

2. Results and discussion

2.1. Chemistry

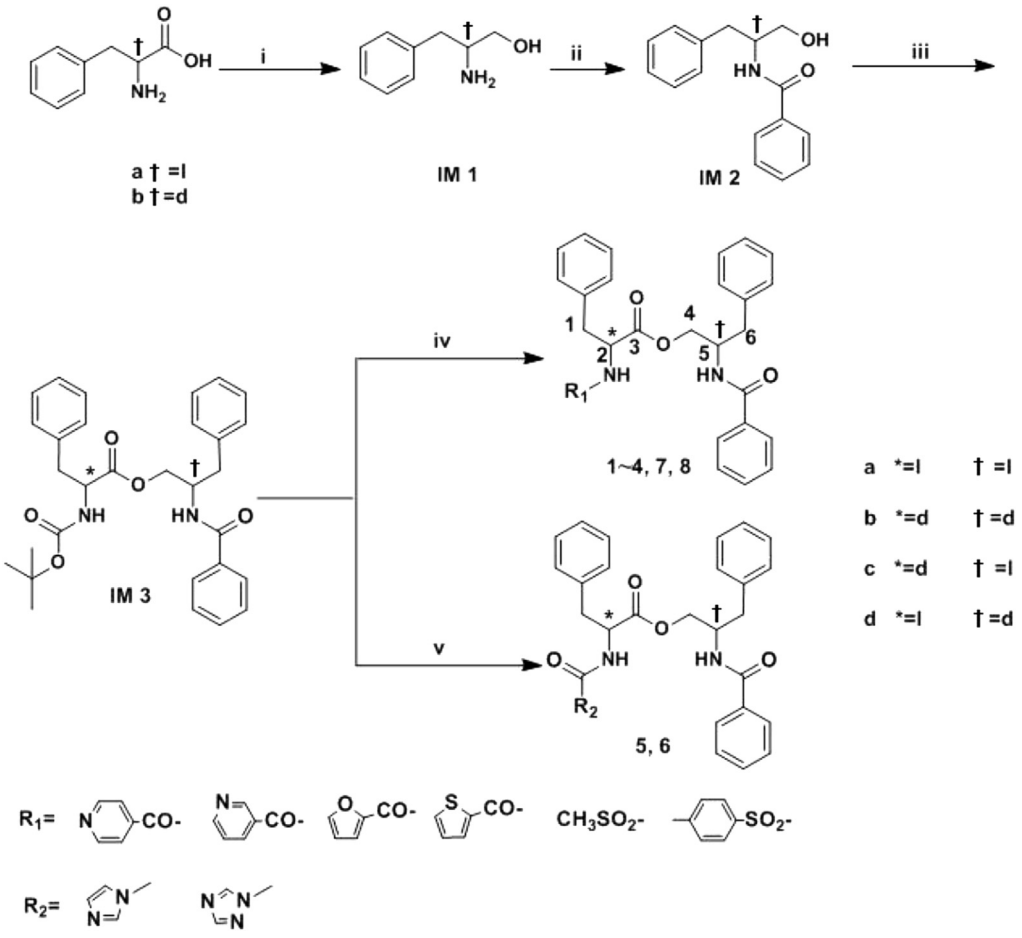
The synthesis of novel asperphenamte derivatives **1**–**8** has been accomplished as outlined in Scheme 1. Starting from optically pure phenylalanine, phenylalaninol **IM1** was prepared according to the method developed by McKennon [20]. *N*-benzoyl-phenylalaninol **IM2** was synthesized by the method of Lewanowicz [21]. Condensation of optically pure *N*-Boc-phenylalanine with **IM2** was promoted by 1, 1'-carbonyldiimidazole (CDI) to afford the key intermediate **IM3**. After the removal of the Boc group by use of 1.5 mol/L HCl (g)/ethyl acetate, **1**–**4, 7** and **8** were directly obtained through acylation reaction. Furthermore, the reaction of deprotected product with triphosgene and imidazole or 1,2,4-triazole in the presence of Et_3N afforded compounds **5** and **6**.

2.2. Biological activity and structure-activity relationships (SAR)

All of these analogs were screened *in vitro* for antitumor activities against MCF-7, HeLa and BEL-7402 cells using the standard MTT method, and cisplatin and **OBA**, the most potent compound in our previous work [13], were used as the positive control. The results were presented in Table 1.

In heterocycles derivatives, almost all *(S, S)* and *(S, R)* configuration compounds displayed no growth inhibitory effect. 2-Furyl and 2-thienyl analogs were inactive with an average IC_{50} value greater than 100 μM. **1c** and **2c** with *(R, S)* configuration, 4-pyridyl and 3-pyridyl derivatives, showed slightly more potency than cisplatin against MCF-7 and BEL-7402 cells. The cytotoxicity of **1c** and **2c** was approximately equal. Imidazolyl and 1, 2, 4-triazolyl derivatives showed similar growth inhibitory effect. And imidazolyl and 1, 2, 4-triazolyl substitution type led to a three to four fold decreased activity compared with **1c**.

In sulfonyl derivatives, with the exception of mesyl derivative with (*S, R*) configuration, other derivatives displayed moderate to excellent growth inhibitory effect. The growth inhibitory activity against three cancer cell lines for tosyl derivatives was superior to mesyl derivatives. Tosyl derivative **8c** showed the greatest potency among tested compounds against three cell lines. The cellular potency of **8c** against MCF-7 and BEL-7402 cell lines was 3.0 and 1.6-fold more potent than that of cisplatin, respectively. **8c** exhibited a similar



Scheme 1. Reagents and conditions: (i) H_2SO_4, $NaBH_4$, I_2, THF, reflux, 98%; (ii) benzoyl chloride, K_2CO_3, MeOH, rt, 95%; (iii) N-Boc-(L or D)-phenylalanine, CDI, $CHCl_3$, rt, 65%; (iv) (a) 1.5 N HCl/AcOEt, rt, 76%; (b) Different acyl chlorides and sulfonyl chlorides, Et_3N, CH_2Cl_2, rt, 92–94%; (v) (a) 1.5 N HCl/AcOEt, rt, 76%; (b) Triphosgene, imidazole or 1,2,4-triazole, Et_3N, CH_2Cl_2, rt, 67–72%.