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Discovery of novel AHLs as potent antiproliferative agents

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ABSTRACT

Three series of novel AHL analogs were synthesized and evaluated for their *in vitro* cytotoxic activity against four human cancer cell lines. The SARs investigation indicated that AHLs with a terminal phenyl group, especially those with the chalcone scaffold had remarkably enhanced cytotoxicity than those with the hydrophobic side chains. Besides, some of these compounds were much more potent than 5-Fu and natural OdDHL. Through the detailed SARs discussions, we found that compounds **10a-k** and **14** with the 4-amino chalcone scaffold showed excellent inhibition against all the tested cancer cell lines and were much more potent than 5-Fu and AHLs. Such scaffold may act as a template for further lead optimization. Compound **10i** with a 3, 4, 5-trimethoxy group was the most potent one against all the tested cancer cell lines. Flow cytometry analysis indicated that analog **11e** induced the cellular apoptosis and cell cycle arrest of MCF-7 cells at G2/M phase in a concentration-and time-dependent manner.

1. Introduction

N-Acyl homoserine lactones (AHLs) have been identified to be responsible for the largest proportion of quorum sensing signals of Gram negative bacteria [1]. *N*-(3-oxododecanoyl)-L-homoserine lactone (OdDHL, Fig. 1), as the well-known quorum sensing signal of *Pseudomonas aeruginosa*, was reported to play a critical role in the infection caused by *P. aeruginosa* [2] and to be able to interfere the eukaryotic system. Besides, numerous studies have showed that OdDHL can induce apoptosis of human breast cancer cell lines through ablating the activity of the signal transducer and activator of transcription protein 3 (STAT3) with little effect on normal breast cells and serve as *r*TSβ mimics down-regulating thymidylate synthase, inhibiting the growth of human colorectal cancer cells (H630) and enhancing the anti-tumor activity of 5-fluorouracil, taxol and tomudex [3,4]. In immunity, OdDHL has been proved to be capable of stimulating the production of IL-8 and inducing apoptosis in specific host immune cells [5,6]. OdDHL and its analogs, as the anticancer agents, have been extensively studied. For example, phenacylhomoserine lactones presented potent anticancer activity and minimum quorum sensing activation [7], and acridine-based AHL analogs showed excellent antiproliferative activity against human oral squamous carcinoma cell lines and even induced radiation-sensitizing effects on Ca9-22 cells and polyploidy in SAS at the concentration of 21.2 μM [8,9].

Dithiocarbamates have received considerable attention for their excellent biological activities and abundance in nature. The natural Brassinin (Fig. 1) has been indentified as potent antifungal agent and moderate inhibitor of cancer immunosuppression target Indoleamine 2, 3-dioxygenase (IDO). The dithiocarbamate group was found to be crucial for the cytotoxic activity [10]. Besides, the dithiocarbamate motif has always been used as a linkage to combine different biologically active scaffold to design new chemical entities [11–14]. Very recently, our group have reported a series of selective LSD1 inhibitors, which can inhibit gastric cancer cell growth, invasion and migration (Fig. 1) [12].

Chalcones and their derivatives have been reported to possess various biological activities [15–22], the anticancer activity, in particular, has received significant attention in last decades [16–18,20,21,23]. In addition to the α, β-unsaturated carbonyl scaffold [20], the amino group of 4′-amino chalcone was also found as the promising site for further modifications to obtain more biologically promising chalcone derivatives with high efficacy and selectivity [18,21].

Inspired by the above-mentioned findings and in continuation with our previously efforts toward finding novel anticancer agents

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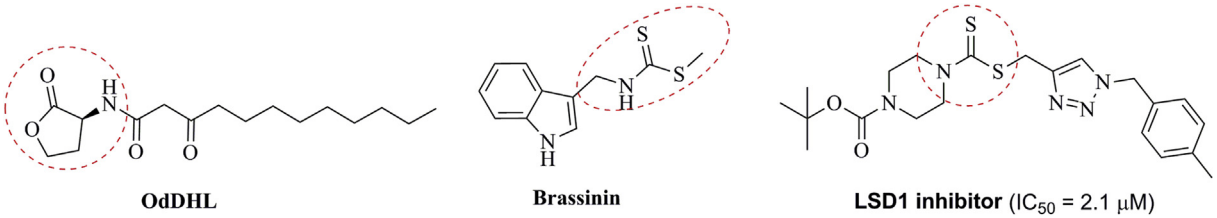


Fig. 1. Structures of OdDHL, Brassinin and LSD1 inhibitor previously reported.

[12–14], we herein report the identification of novel antiproliferative AHL analogs with the chalcone and homoserine lactone scaffold linked by the dithiocarbamate group through extensive SARs investigations. Besides, the promising 4′-amino chalcone scaffold with potent cytotoxicity was first discovered.

2. Results and discussion

2.1. Chemistry

L-homoserine lactone hydrochloride salt **1** was prepared from L-methionine following the literature reported method [24]. Compounds **3a-r** were obtained from the corresponding anilines through the well established acylation reaction. AHL analogs **4a-r** and **6a-g** were then synthesized from compound **1** and corresponding intermediates **3a-r** and **2a-g** via a three-component one-pot reaction following our previously reported method [23] (Scheme 1).

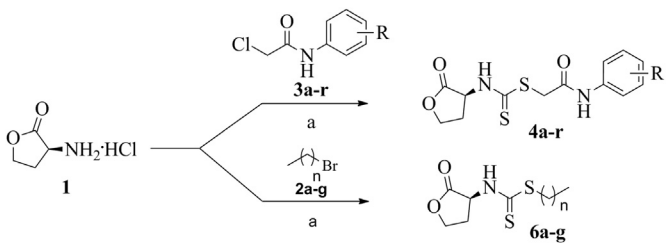
The condensation of 4-amino acetophenone and **8a-k** in the presence of NaOH efficiently gave 4'-amino chalcones **9a-k**, which then reacted with chloroacetyl chloride, generating acylated 4'-amino chalcones **10a-k**. With these compounds in hand, analogs **11a-g** were efficiently synthesized via a three-component one-pot reaction (Scheme 2). To further explore the SARs, compound **14** with an additional double bond was also synthesized from cinnamaldehyde **12**.

Natural OdDHL was synthesized according to the literature reported method [25]. Meldrum's acid **17** was easily formed via the reaction of malonic acid **15** with acetone **16**, followed by the coupling reaction with *n*-decanoic acid in the presence of DCC and DMAP, giving compound **19**, which reacted with compound **1** to give OdDHL in the presence of Et_3N (Scheme 3).

2.2. Biological evaluation

2.2.1. Cytotoxic activity

The IC_{50} values (concentration required to inhibit the proliferation of cancer cells by 50%) for all obtained AHL analogs **4a-r**, **6a-g**, **11a-g**, **14** and substituted amino chalcones **10a-k** against four human cancer cell lines including MGC-803 (human gastric cancer cell line), MCF-7 (human breast cancer cell line), EC-9706 (human esophageal cancer cell line) and SMMC-7721 (human hepatocellular carcinoma cell line) were determined using MTT assay [26], and OdDHL and 5-Fluorouracil were both used as positive controls.



Scheme 1. Synthesis of AHL analogs **4a-r** and **6a-g**. Reagents and conditions: (a) CS_2, $Na_3PO_4 \cdot 12H_2O$, acetone, rt.

The SARs were fully explored by changing the substituents attached to the dithiocarbamate group. The *in vitro* inhibitory activity was summarized in Table 1. It is evident that compounds **6a-g** with hydrophobic carbon chains showed weak or no inhibition against the tested cancer cells. Specifically, no cytotoxicity was observed for compounds **6a-e** (n < 8), compounds **6f-g** with relatively prolonged side chains (n = 11 and 13) showed slightly improved inhibitory activity. This indicated that the hydrophobic chain may be not beneficial for the activity.

Compounds **4a-r** with different terminal phenyl groups were then synthesized and evaluated for their cytotoxicity. Interestingly, most of these compounds showed improved inhibitory effect against the tested cell lines compared to compounds **6a-g**. Compounds **4a-r** had weak or no activity against EC-9706 and SMMC-7721 cells. However, an increased inhibition against MGC-803 and MCF-7 cells was observed. It is worth noting that compounds **4l-n** showed excellent inhibition against MCF-7 cells (IC_{50} = 3.13, 5.46 and 5.83 μM, respectively) and were much more potent than OdDHL and 5-Fu (IC_{50} = 37.31 and 12.24 μM, respectively). For MCF-7 cells, analogs with electron-withdrawing groups showed better cytotoxicity than those with electron-donating groups. In particular, compounds with electron-withdrawing substituents at *meta*-position of the phenyl ring contributed more to the cytotoxicity, such as analogs **4l** (3-CF_3) and **4m** (3-NO_2). Analogs with the same electron-withdrawing group at *meta*-position showed better cytotoxicity against MCF-7, while weaker cytotoxicity was observed when the group was replaced at the *ortho*-position, like **4f** (3-Cl) >**4b** (4-Cl) >**4h** (2-Cl). Analogs substituted by halogen atoms F, Cl and Br at the same position on the phenyl ring exerted an activity sequence of **4n** (4-F) >**4b** (4-Cl) >**4c** (4-Br). For MGC-803 cells, compounds **4a-r** showed moderated inhibition with the IC_{50} values ranging from 17.88 μM to >128 μM. However, most of them were much more potent than OdDHL (IC_{50} = 101.83 μM). From the biological data of compounds **6a-g** and **4a-r** against MGC-803 cells, we can conclude that compounds with a terminal phenyl group have better cytotoxicity than those with a hydrophobic side chain regardless of the length.

This interesting finding promoted us to further explore the SARs by incorporating the chalcone scaffold into our molecules (compounds **11a-g**). A significant enhanced cytotoxicity against the tested cancer cell lines was observed, especially for EC-9706 cells, compared to compounds **6a-g** and **4a-r**. Specifically, compounds **11e** and **11g** represented excellent inhibitory effect against MCF-7 cells (IC_{50} = 5.57 and 2.67 μM, respectively) and were much more potent than OdDHL and 5-Fu. Besides, compound **11a** also had excellent inhibition against MGC-803 cells with the IC_{50} value of 6.90 μM. This finding indicated that the chalcone scaffold played an