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Synthesis and biological evaluation of lovastatin-derived aliphatic hydroxamates that induce reactive oxygen species

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ABSTRACT

Some hydroxamate compounds induce cancer cell death by intracellular reactive oxygen species (ROS). This study introduced the hydroxamate core into lovastatin, a fungus metabolite clinically used for the treatment of hypercholesterolemia. The resulting compounds were evaluated for the activity for inducing ROS production. Most compounds exhibited higher activity than original lovastatin. Of these compounds, compound **3c** had the most potent activity. Test of cytotoxicity in a panel of human cancer cell lines indicated compound **3c** had activities superior to cisplatin in prostate cancer PC-3 cells and breast cancer T47D cells. In contrast, it in amounts up to 40 μM had a much lower cytotoxic effect on normal human IMR-90 cells. Further profiling of cell cycle progression, cell apoptosis, and DNA damage activated checkpoint signaling pathway revealed the important role of compound **3c**-mediated cytotoxicity in ROS generation.

Reactive oxygen species (ROS), which are chemically reactive molecules containing oxygen, have an essential role in maintaining the homeostasis of physiological function.[1] Moderate ROS levels regulate cell growth and differentiation, control enzyme activity, and modulate inflammation.[2,3] In contrast, excess ROS cause oxidative damage leading to cell death by reacting with intracellular biomolecules such as DNA, proteins and lipids.[4] Many tumor cell types reportedly have higher ROS levels compared to their normal counterparts.[5–7] Enhanced ROS production in cancer cells can cause ROS levels to reach the toxic threshold at which malignant cells death is preferentially induced. Therefore, agents that promote ROS production may have therapeutic applications in anticancer strategies.[8,9]

Lovastatin (**1**), a fungal metabolite originally isolated from *Aspergillus terreus* and from *Monascus rubber*,[10–12] is widely used to treat hypercholesterolemia. It competitively inhibits 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, which catalyzes the reduction of HMG-CoA into mevalonate. This effect then lower cholesterol.[13] Lovastatin (**1**) also blocks the synthesis of mevalonate-related downstream isoprenoids such as geranylpyrophosphate and farnesylpyrophosphate, both of which bind to Ras and its related proteins, and thus perturbs the function of G-proteins.[14,15] These studies suggest that lovastatin (**1**) may have various cell physiological activities that affect cell growth, including cell proliferation, apoptosis, invasion and differentiation.[16–19] These physiological activities are further described below. In several tumor cell types, lovastatin (**1**) is known to inhibit proliferation, induce apoptosis, and arrest the cell cycle in G1/S phase.[15,20] Studies reveal that lovastatin (**1**) combined with chemotherapeutic drugs synergistically sensitizes cancer cells to anticancer agents in several tumor cell lines as well as in vivo models.[21–25] Despite its many anticancer studies reported, attempts for applying chemical synthesis to improve lovastatin activity are few.[26] Various hydroxamate-containing compounds reportedly can not only inhibit histone deacetylase (HDAC), but also induce intracellular ROS production. The combinatorial effect causes cancer cell death.[27–29] To enhance the antiproliferative activity of lovastatin (**1**), the hydroxamate motif that is associated with the

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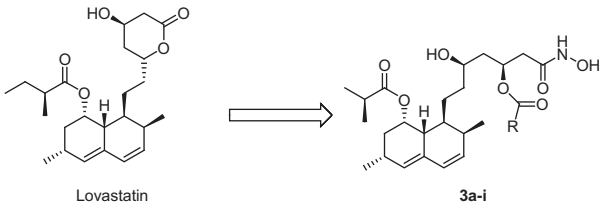
ROS production was experimentally incorporated into the chemical structure of lovastatin (Scheme 1). We hypothesized that this modification would enhance the ROS promoting activity of lovastatin (**1**) in tumor cells. Evaluations of ROS-inducing ability of all synthesized compounds (**3a–j**) showed that compound **3c** significantly induced ROS production in cancer cells, but had no HDAC enzyme inhibitory activity. Notably, the cytotoxicities of compound **3c** in several cancer cells were higher than those of lovastatin (**1**). This study then examined how compound **3c**-mediated ROS production affects cell cycle progression, cell apoptosis, and DNA damage in the checkpoint signaling pathway.

Lovastatin-based aliphatic hydroxamates were synthesized as shown in Scheme 2. Lovastatin (**1**) reacted with the appropriate acyl chlorides gave **2a–i**, respectively. Reaction of compounds **2a–i** with NH_2OH provided the corresponding hydroxamates **3a–i**. Basic saponification of lovastatin (**1**) in the presence of LiOH and subsequent lactonization gave **4**. Compound **4** was reacted with NH_2OH to provide **3j**.

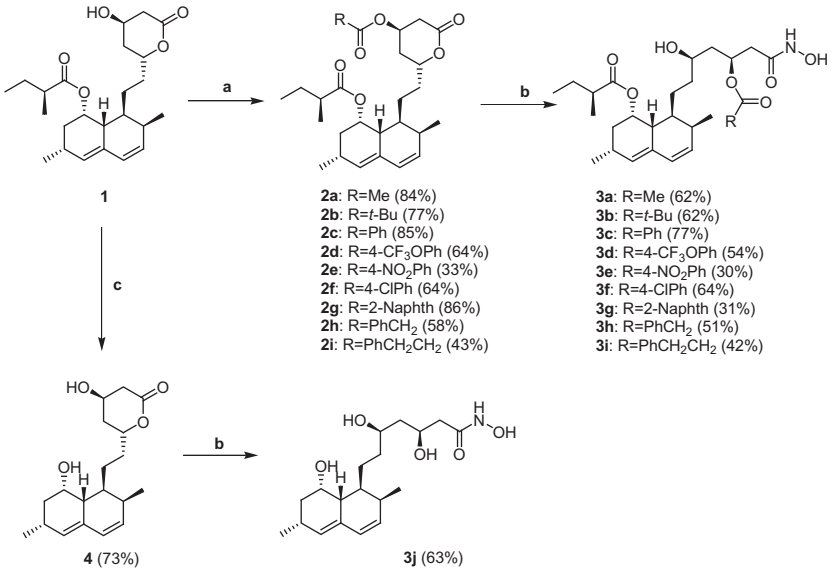
Figure 1 shows that flow cytometric analysis was performed using DCFH-DA fluorescent probe in human breast cancer MDAMB-231 cells to test all lovastatin-derived compounds **3a–j** synthesized in this study for activity that induced ROS production. Additionally, two positive controls doxorubicin[30] and cisplatin[31] that reported increased ROS production in cells were included in the test. Comparisons of compounds **3a–j** and lovastatin indicated that the activities of all compounds were improved compared to that of original lovastatin. However, these compounds exhibited no HDAC-inhibiting activity (Fig. 1S). Most compounds, except for compound **3j**, had higher ROS production activity than did cisplatin. Notably, the activities of compound **3c** and **3g** were strongest. The activities of benzoyl substituted series **3c–g** were higher than those of both compounds **3a** with acetyl group and **3b** with *t*-butanoyl group, which suggested that the benzoyl moiety positively contributed to activity. Comparisons of substituted benzoyl aliphatic hydroxamates **3d–g** showed that compound **3g** had the highest potency. However, its potency was comparable to that of compound **3c**, which indicated that the substituent on phenyl ring only slightly increased ROS production. Next, compounds **3h–i** with one to two carbons chain-length of linker attached to phenyl ring were synthesized. These compounds had equally potent activities. Moreover, compound **3j** had only weak activity. These experimental results speculated that the weak ROS-inducing activities of compounds **3a, 3b,** and **3j** were possibly caused by their poor cell membrane permeability and the weak electron-donating ability of the substituent on the β-position of these tree compounds.

The results of growth inhibition against human prostate PC-3 cells indicated that the cytotoxicities of three of these compounds, **3c**, **3h** and **3i**, equaled or exceeded that of lovastatin (Table 1S). Moreover, their activities were much higher than that of cisplatin. In particular, the antiproliferative activity of compound **3c** was highest.

In further experiments to identify potential anti-breast cancer agents, compound **3c** with significant growth inhibition was selected for the evaluation of antiproliferative activity against a panel of human breast cancer cell lines such as MDA-MB-231, MCF7 and T47D cells using cisplatin as a positive control. In T47D and MCF7cancer cell lines, compound **3c** exhibited higher cytotoxicities (IC_{50} = 30–40 μM) than did lovastatin (IC_{50} > 40 μM) (Fig. 2A–C). Compared to cisplatin, the antiproliferative activities of compound **3c** against MDA-MB-231 and MCF7 were comparable, but its activity against T47D cells was higher. In contrast, compound **3c** even in amounts up to 40 μM exhibited lower cytotoxicity for normal IMR-90 cells compared to cisplatin (Fig. 2D). These experimental results indicated that compound **3c** may have the therapeutic index superior to cisplatin.



Scheme 1. Design of lovastatin-derived hydroxamates **3a–i**.



Reagents and condition : (**a**) Ac_2O, Pyr, RT, for **2a**, ; RCOCl, Pyr, $PhCH_3$, RT, for **2b-i**; (**b**) 50% NH_2OH, THF, N_2, RT; (**c**) (1) LiOH, MeOH-THF, Δ; (**2**) $PhCH_3$, Δ.

Scheme 2. Reagents and condition: (**a**) Ac_2O, Pyr, RT, for **2a**; RCOCl, Pyr, $PhCH_3$, RT, for **2b–i**; (**b**) 50% NH_2OH, THF, N_2, RT; (**c**) (1) LiOH, MeOH–THF, Δ; (**2**) $PhCH_3$, Δ.