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레티노이드 효과를 가지는 합성물에 의한 피부재생 효과

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Skin Rejuvenation by Novel Synthetic Compound Containing Retinoidal Activity

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요 약: 레티노이드 효과가 나타나는 것을 목적으로 하여 다양한 히드록삼산 유도체를 합성하였고, 레티노익산 수용체에 대한 전사 활성을 측정하여 스크리닝하였다. 합성된 화합물 중에서 N-(4-N-hydroxycarbamoyl)phenyl) [4-(tert-butyl)phenyl] carboxamide (**2f**)가 수용체에 대한 가장 강력한 적합성을 나타내었다. 히드록삼산 구조에서 산도를 나타내는 히드록시기는 쉽게 이온화하여 음이온을 형성한다. 형성된 히드록삼산의 음이온은 레티노익산의 음이온과 유사한 역할을 한다. 이와 같이 히드록삼산 화합물이 레티노이드 효과를 나타내는 예는 이전에 알려진 바가 없다. 화합물 **2f**의 레티노이드 효과는 레티노이드와 연관된 유전자의 발현 증가 효과로 한번 더 검증하였다. 노화 원료로서의 가능성을 확인하기 위해서 MMP-1의 발현 억제능을 레티노익산, 레티놀과 비교하여 살펴보았다. 10 μM 농도에서 화합물 **2f**는 MMP-1의 발현을 억제하였다. 이와 같은 결과로 화합물 **2f**는 항노화 원료로서의 가능성을 가지고 있다.

Abstract: Several hydroxamic acid derivatives are synthesized to observe retinoidal effect and transactivation potential for RARα/γ is screened. Among the synthesized compounds, N-(4-N-hydroxycarbamoyl)phenyl) [4-(tert-butyl)phenyl] carboxamide (**2f**) showed the best compatibility for potent RARα/γ. The acidic hydroxy of hydroxamic acid was easily deprotonated to form an enolate. A formed enolate has a similar role like that of all-trans-retinoic acid. This is the first example of the hydroxamic acid derivative with retinoidal activity. The retinoidal activity of **2f** was further confirmed by enhancing activity for the expression of retinoid-responsive genes. To evaluate the possibility for anti-aging agent, effect on the expression of MMP-1 was measured comparing with all-trans-retinoic acid and retinol. At 10 μM treatment, compound **2f** inhibited the expression of MMP-1. These results suggest that new hydroxamic acid derivative **2f** could be used as a promising anti-aging agent.

Keywords: *hydroxamic acid, retinoic acid, receptor, MMP-1, aging*

1. Introduction

Photo-aging is mainly due to ultraviolet irradiation of sunlight, which overwhelmingly contributes to a premature aging[1]. As skin is increasingly exposed to UV-irradiation, this increases risk for photo-oxidative damage to skin with long term detrimental effects, characterized by wrinkles[2], loss of skin tone[3], and resilience[4]. This irreversible aging process results from UV induction of matrix metalloproteinases (MMPs)[5,6] that degrade skin components such as collagen, elastin, and laminin etc. There are two pathways for the regulation of MMP series. One is inhibition of MMP activity[7]. The other is reducing the expression of MMP series[8]. Although various agents have been developed, all-trans-retinoic acid (ATRA)[9] is most potent regulating agent for the expression of MMP series. ATRA is known to be effective against various skin diseases and are now being considered as potential drugs for treatment of several cancers. However, direct topical treatment with ATRA causes severe side effects, such as skin irritation [10], cosmetic use of ATRA is not allowed. A number of synthetic retinoids have been developed but there is still a need to find new retinoidal compound without compromising skin irritation.

Here, we designed and synthesized novel new retinoidal

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compound, which possesses a hydroxamic acid moiety instead of the carboxylic acid group of ATRA. The hydroxamic acid is widely known to be as well as a metal ion chelator and an inhibitor for MMP-1 activity. However, it has not been used as an alternative of carboxylic acid. Recently, carboxylic acid of ATRA has been replaced by various functional groups such as phenol, thiazolidinone, tetrazole, and tropolone moiety[11]. Retinoidal activities were shown in their structure. In this paper, retinohydroxamic acid compounds were synthesized and their retinoidal activities were screened by tansactivation potential for RARα/γ. After screening these compounds, compound **2f** was selected by its potent RARα/γ agonistic activity. And then its biological activities were evaluated for example RAR-responsive gene 1, RAR-responsive gene 2, and MMP-1.

2. Result and Discussion

2.1. Chemistry

Hydroxamic acid is widely used to be a metal ion chelator. The acidic hydroxy of hydroxamic acid can be transformed to be enolate, which can be bounded to metal ion. Here we are interested in the character of hydroxamic acid as an alternative of carboxylic acid (Figure 1).

The synthesis of retinohydroxamic acid derivatives (**2a-2i**) is illustrated in Scheme 1.

4-substituted benzoic acid, adamantanecarboxylic acid or mono-methyl terephthalate were refluxed in thionyl chloride to convert the carboxylic acid to an acid chloride. This compound was reacted immediately with methyl-4-aminobenzoate, 4-amino phenylacetic acid methyl ester or adamantamine in pyridine to produce the corresponding amide derivatives. The ester group was hydrolyzed under standard condition (NaOH, ethanol) to produce corresponding acid. The acid was reacted with ethylchloroformate and N-methyl morpholine in THF to convert the carboxylic acid to an anhydride. This compound was reacted immediately with hydroxylamine to produce the corresponding retinohydroxamic acid derivative **2a-2i**

2.2. Transactivation Potential for RARα/γ

Compounds **2a-2i** were evaluated for their transactivation potential for RARα and RARγ using transient transfection experiment employing RARE-tk-Luc reporter gene encoding RAR response element (RARE). Transactivation potential for RARs was the evidence for retinoidal agonistic activity of hydroxamic acid derivatives. As shown in Figure 2, compound **2a** showed moderate activity for RARγ, however, very little effect on RARα. The introduction of methyl at *para* position afforded **2b**, which showed similar activity for RARα and RARγ. But ethyl, propyl, and butyl analogs showed decreasing transactivation potentials, indicating that a linear lipophilic group is not preferred in the ligand binding pocket that accommodates hydrogen and methyl group. Compound **2f**, containing *tert*-butyl group, exhibited potent activity. However, compound **2g** is structurally less rigid than **2f** that showed loss of activity.

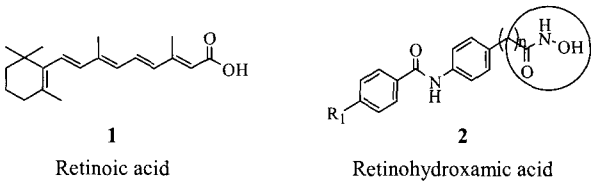
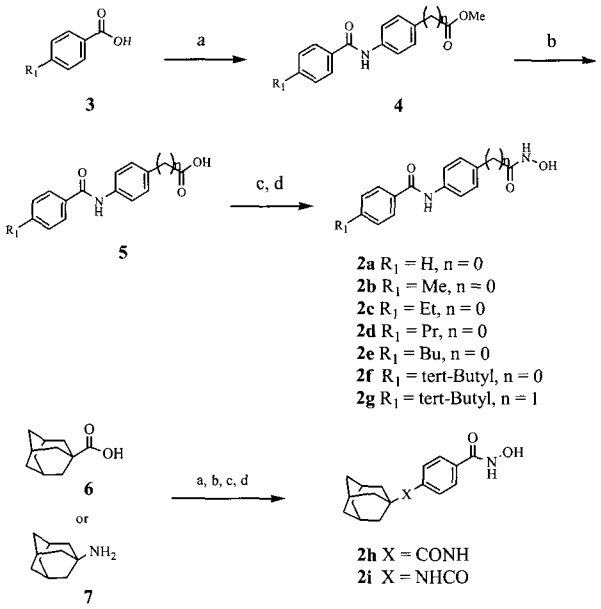


Figure 1. Structure of retinohydroxamic acid.



Reaction conditions; (a) $SOCl_2$, reflux; (b) methyl 4-amino benzoate or 4-aminophenylacetic acid methylester, MC, TEA; (c) NaOH, EtOH; (d) ethyl chloroformate, N-methylmorpholine, THF; (e) hydroxylamine HCl, TEA, DMF

Scheme 1. The synthesis of retinohydroxamic acid derivatives (2a-2i).

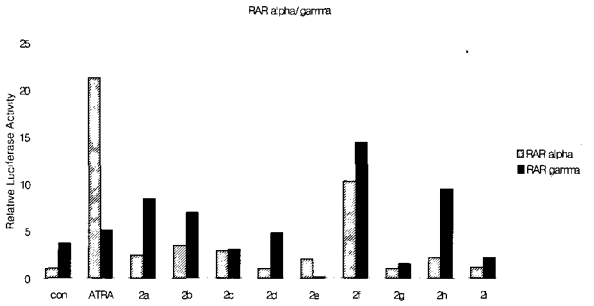


Figure 2. Effects of hydroxamic acid derivatives on the transcriptional activity of RARα and RARγ. All compounds were tested at 10 μM except of ATRA (0.1 μM).

These finding suggested that the size of substituent of the *para* position and planar structure were critical for RAR agonistic activity. We synthesized compound **2h** and **2i** that possess adamantyl moiety instead of *tert*-butyl phenyl group in the structure of **2f**. Compound **2h** showed potent activity for RARγ. But compound **2i** showed low activity for RARα and RARγ. After screening RARα/γ agonistic activity, compound **2f** was selected as a candidate for anti-aging agent, since RARγ is known to be most abundant isotype in the skin.

2.3. Effects on Expressions of Retinoid-Responsive Genes

To assure the retinoidal activity of compound **2f**, retinoid-responsive genes were monitored in compound **2f** treated HaCaT cells using real-time RT-PCR. As shown in Figure 3, compound **2f** treatment at 10 μM resulted in 3.6-fold increase in retinoid-responsive gene-1 mRNA-level. Similarly, mRNA-level of RAR-responsive gene 2 was also increased 2.4 fold, respectively after compound **2f** treatment at the same concentration. The potency of compound **2f** on the expression of retinoid-responsive genes was dose dependant. ATRA (0.1 μM) and ROL (10 μM) showed the similar expression patterns.

2.4. Effects on Expressions of MMP-1

To evaluate the anti-aging effect of compound **2f**, we examined the inhibitory effects on expression of MMP-1. The results are summarized in Figure 4. The level of secreted MMP-1 was measured in cell media by an enzyme-linked immunosorbent assay (ELISA). Twenty four hours of exposure to 10 μM compound **2f** decreased the levels of MMP-1 by about 50%, compared to UV-irradiated control.

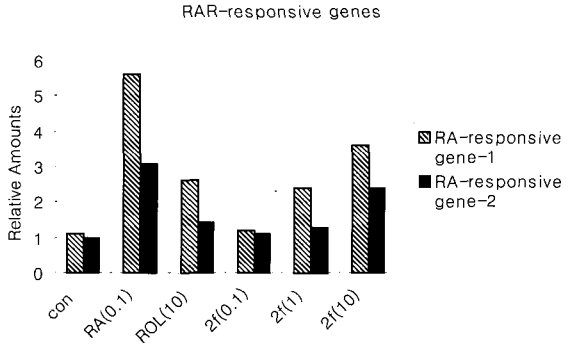


Figure 3. Effect of compound 2f on the transcriptional activity of RAR-responsive genes.

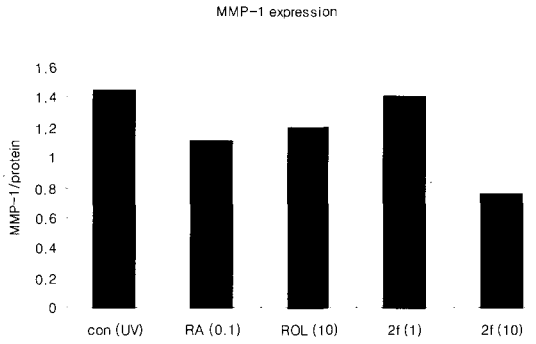


Figure 4. Effect of compound 2f on the expression of MMP-1.

3. Conclusion

We synthesized new hydroxamic acid derivatives as retinoids on the assumption that the hydroxamic acid moiety may show similar behavior of carboxylic acid in ATRA structure. Retinoidal activities of synthesized compounds were measured by reporter gene assay. After testing, we propose that compound N-(4-N-hydroxycarbamoyl)phenyl) [4-(tert-butyl)phenyl] carboxamide (**2f**) is a good candidate of anti-aging agent due to its potent RARα/γ agonistic activity. Other results provide evidence that **2f** exhibit retinoidal properties expressed either by its enhancing activities for RAR-responsive gene-1 and RAR-responsive gene-2. We have also shown that inhibitory activity of **2f** for the expression of MMP-1. This fact suggests interesting perspectives for the use of compound **2f** in the treatment of skin aging.

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