Development of Novel Pyrone Derivative Retaining Retinoidal Anti-aging Activity with Low Skin Irritation

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Abstract

New pyranone derivative, 2-((3E)-4(2H,3H,-benzo[3,4-d]1,3-dioxolan-5-yl)-2-oxo-but-3-enyloxy)-5-hydroxy-4H-pyran-4-one (Seletinoid G^{TM}), was designed as a novel retinoid on the assumption that the pyranone ring may mimic the carboxylic acid moiety in retinoid structure. The enolic hydroxy of pyranone at five position was easily deprotonated to form an enolate. The role of enolate was similar to that of carboxylic acid. To evaluate the value of Seletinoid G as an anti-aging ingredient, various tests were performed for example inhibitory effect for MMP-1 expression, anti-oxidative activity, procollagen synthesis in hairless mouse and primary skin irritation. The result of this study suggested that our new synthetic retinoid could be used as a safe material for anti-aging cosmetics.

Key word retinoid, MMP-1 expression, antioxidant, procollagen, anti-aging, cosmetics

Introduction

Retinoids are natural and synthetic derivatives of retinoic acid (RA). The biological activities of retinoid are mediated by binding to and activation of the retinoid acid receptors (RARs), followed by modulation of target gene transcription by complex.[1] The structure of retinoid consists of main two parts, carboxylic acid and hydrophobic group. The binding affinity for RAR requires a carboxylic acid moiety and an appropriate hydrophobic group. These two groups are linked various functional such as ester, stilbene, amide and so on.[2] The role of linker is also critical for the appearance of binding affinity. Recent work on the design of synthetic retinoids and the availability of 3D structural information have revealed the structure requirements for subtype selectivity.[3] RA is the natural ligand for the RARs. It showed broad biological activities such as anti-aging,[4] anticancer,[5] anti-acne[6] and psoriasis.[7] Although its potent efficacy was useful, the side effect of RA is problem that was originated the non specific receptor binding.[3] Many scientists have been developed synthetic retinoid to reduce the side effect of RA. One of main research stream is the development of subtype specific ligand for RARs. In human skin, RARγ is predominant RAR, the rest being RARα.

RARγ is involved in anti-aging and skin disease like acne and psoriasis. Due to the important role of RARγ, there is an ongoing interest in the synthesis RARγ specific ligands which can be used as cosmetic for anti-aging. Here, we designed and synthesized novel new retinoids, which possesses an enolic hydroxy of 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one instead of the carboxyl group of RA. The 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one is widely known to be as well as a skin depigmenting agent[8] and a metal ion chelator.[9] The structure of 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one is γ-pyrone having enolic hydroxy group, easily can be transformed to be enolate. The acidic character of 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one is similar to the carboxylic acid of RA and benzoic acid of retinobenzoic acid. Recently, tropolone moiety was used as an alternative of carboxylic acid, which also express a retinoidal activity.[10] In this paper will be presented a novel derivative of 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one (Seletinoid G^{TM}), shows a RARγ selectivity vs RARα, inhibition of MMP-1 expression, anti-oxidative activity, procollagen synthesis with low skin irritation

Experimental

Materials

All reagents were used without further purification. Kojic aicd, 3,4-(methylenedioxy) cinnamic acid, KOH and $SOCl_2$ were purchased from Sigma Chemical Co (USA)

Structure and Synthesis of Seletinoid G

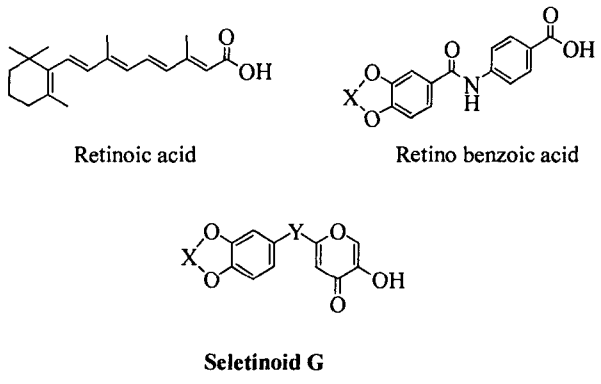
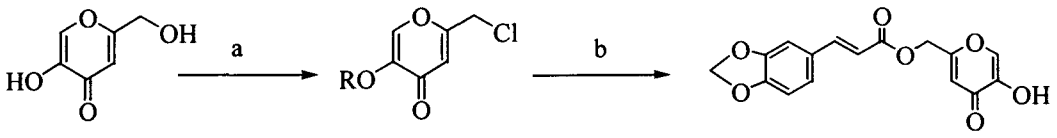


Fig. 1. Structure of Seletinoid G

A synthetic route for Seletinoid G is outlined in Fig 2. The primary hydroxy of

5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one was easily transformed to chloride by treating with $SOCl_2$. The chloride was reacted with pottsium salt of 3,4-(methylenedioxy)cinnamic acid to give Seletinoid G.



Seletinoid G

(a) $SOCl_2$, DMF; (b) Potassium salt of 3,4-(methylenedioxy)cinnamic acid, DMF

Fig. 2. Synthetic pathway of Seletinoid

Assay of RARγ over RARα

CV-1 cells were cotransfected with expression vectors for either human RARα or RARγ and with the ERE-tk-Luc reporter plasmid. They were then grown for 24hours in the presence of RA and Seletinood G. Luc expression was determined using an ELISA technigue.

Cell culture

Human dermal fibroblasts from the foreskin were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 0.48mg/ml glutamine, 100IU/ml penicillin, 50mg/ml streptomycin, and 10% fetal bovine serum. Between the fourth and seventh passage, cells were used for the experiment

Measurement of Secreted MMP-1

Skin fibroblasts were seeded in 48-well plates. Confluent cultures of fibroblasts were irradiated with a high-intensity UVA source (Dermlight cube 401 equipped with UVA filters, Uvatec, Inc.) through the thin layer of PBS in the tissue culture plate. After irradiation, fibroblasts were re-fed with 0.5ml of DMEM without serum and incubated for 48 hours. Interstitial collagenase was measured with the MMP-1 human ELISA system (Amersham Pharmacia Biothch, UK).

Anti-oxidant activity

Lipid peroxides were measured in HaCat cells. One hundred thousand cells were seeded 24-well plates. After 24 hours later, test compounds were added to culture media. After 24 hours later, t-BHT was treated. After 8 hours of culture, the cells were collected and lipid peroxides were measured.

Procollagen synthesis in hairless mice

Female albino hairless mice (Skh:hr-1), 6-8 weeks old, were obtained from Charles River Lavoratories (Wilmington, Mass., USA), grown to 26 weeks, and then used for experiment. Solutions of vehicle (99.9% ethanol: propylene glycol, 7:3 v/v), RA (01.%), ROL (0.1%), Seletinoid G (0.1%) were applied to the dorsal trunk with occlusion for 6 days. Immunohistological analysis of type I pN collagen was performed. Type I pN collagen was detected with mice monoclonal IgG1 antibody raised against the aminoprepeptide region of human type I procollagen. For the histological analysis of the skin, samples were treated with hematoxylin and eosin and examined under light microscopy

Primary skin irritation test

Primary skin irritation test was elicited on the backs of rabbits using Draize's method. Hairs of rabbits were removed and 1% of RA, ROL and Seletinoid G were topically applied on the 2.5cm diametric circle on the right side of back. The left side of back was not treated. After 24 hours or 72 hours later, the degree of irritation was measured according to P.I.I (Primary Irritation Index).

Result and discussion

Assay of RARγ over RARα

The RARγ and RARα selectivity of the Seletinoid G was studied by a reporter cell assay. Seletinoid G has a good selectivity for RARγ. Although the concentration is lower than RA, its potency is similar to that of RA. The transactivation for RARs has been regarded as an evidence for retinoidal activity.

Table 1. Binding assay for RARγ over RARα

Compounds	RARγ	RARα
Retinoic acid (0.1μM)	9398.1	20599.6
Seletinoid G (1μM)	9099.2	1139.5
Seletinoid G (10μM)	8925.4	1518.4

Inhibitory activity of MMP-1 expression

To confirm the relationship between transactivation potential and realistic activity, we tested the inhibition ability of MMP-1 expression. It has been widely accepted result that anti-aging in the skin was mainly originated from the action of MMP-1. The expression of MMP caused a degradation of collagen. RA is well known inhibitor for MMP-1 expression. Seletinoid G showed strong MMP inhibition. Althouh its activity was weaker than that of RA, it was reasonable for anti-aging agent. In cosmetic area, retinol only can be used as an anti-aging agent.

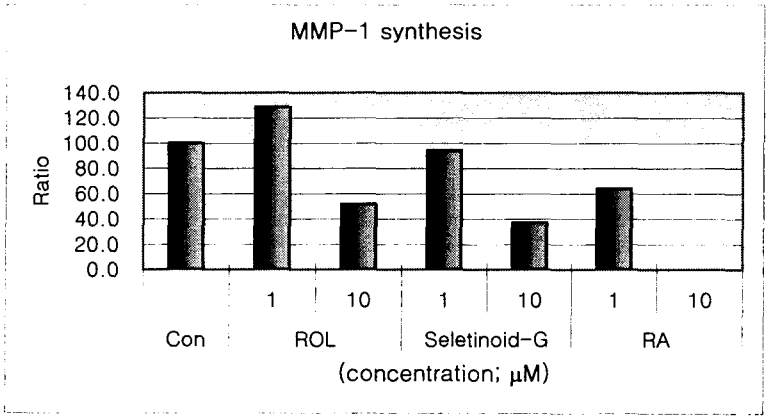


Fig 1. Inhibitory activity of Seletinoid G for MMP-1 expression

Anti-oxidant activity

5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one was expected to have anti-oxidative activity, since it possesses iron-chelating capacity. The anti-oxidative activity of Seletinoid G is evaluated because its structure has 5-hydroxy-2-(hydroxymethyl)-4*H*-pyran-4-one moiety. Seletinoid G reduced concentration of lipid peroxid induced by t-BHT.

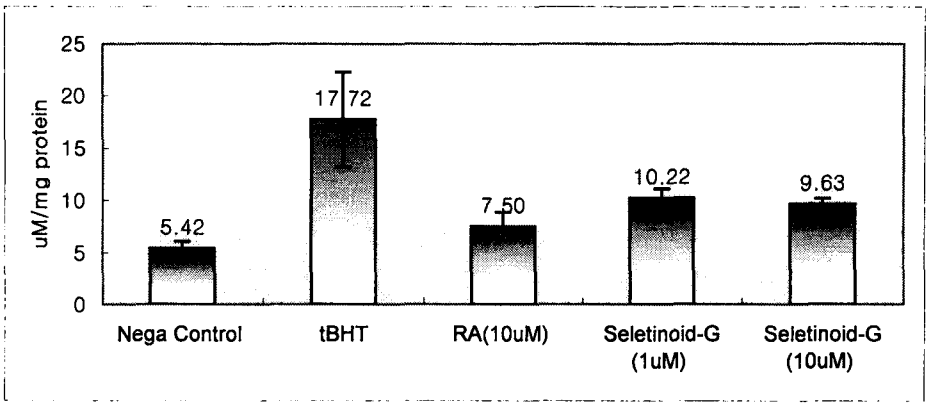
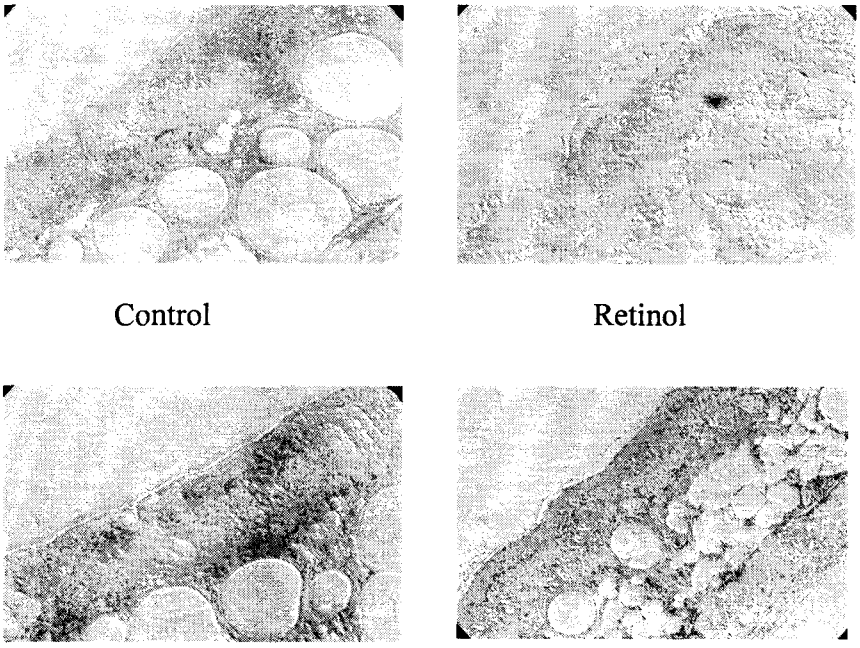


Fig 2. Inhibitory activity of Seletinoid G for the synthesis of lipid peroxides

Procollagen synthesis in hairless mice

The procollagen content was significantly enhanced by topical application of 0.1% Seletinoid G. The activity order is RA>Seletinoid G>ROL>control



Control Retinol

Retinoic acid Seletinoid G

Fig 3. Procollagen sythesis of Seletinoid G, RA and ROL

Primary skin irritation test

We tested primary irritation of Seletinoid G in comparison with ROL and RA. The primary irritation was the main problem of RA in application on the skin. When rabbit was topically applied with seletinoid G, there was no detectable irritation. Thus, Seletinid G could be used as an anti-aging agent without irritation, which is frequently

encountered in various retinoid

Table 2. Primary skin irritation of kojic acid derivatives

Compounds	Irritation P.I.I
Retinoic acid	1.83
Retinol	1.02
Seletinoid G	0.05

Conclusion

Seletinoid G showed potent transactivation potential for RARγ. As a result of transactivation potential, Seletinoid G can be considered as a synthetic retinoid. Seletinoid G showed as well as moderate inhibitory potency for MMP-1 expression and anti-oxidative activity. In MMP-1 expression assay, the activity order is RA>Seletinoid G>ROL. Although its activity was weaker than RA, it was sufficient for anti-aging agent. In cosmetic area, retinol only can be used as an anti-aging agent. The use of RA was not allowed not for activity but for skin irritation. The possibility of Seletinoid G as an anti-aging agent was confirmed in animal model. The increased procollagen was detected in histochemical study. The procollagen synthesis must be caused by the inhibition of MMP-1 expression or anti-oxidative activity. We tested primary irritation of Seletinoid G in comparison with RA and ROL. The primary irritation was the main problem of RA and ROL. In case of Seletinoid G, there was no detectable irritation but RA and ROL showed severe skin irritation. Thus, Seletinoid G could be used as an anti-aging agent without irritation, which is frequently encountered in various retinoid. Even though the low irritation of Seletinoid G is interesting, we have not been able to explain in our studies. Further studies about the reason for low irritation are under study

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