Discovery of natural compounds with anti-melanogenic effect through targeting ATP-P2X7 axis

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Discovery of natural compounds with anti-melanogenic effect through targeting ATP-P2X7 axis

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The Master's Thesis
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree
of Master of Medical Science

Sujin Park

December 2021

This certifies that the Master's Thesis of
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December 2021

ACKNOWLEDGEMENTS

The final outcome of this study required much guidance and inspiration from Professor Sang Ho Oh. I would like to deeply thank him for all his support and encouragement along the completion of this study.

I also owe my profound gratitude to Professor Jinu Lee and Professor Do Young Kim who took keen interest on this study and offered invaluable professional advices and guidance.

I would like to thank Dr. Eun Ju Choi from the College of Pharmacy for discovering the key molecule of this thesis. The natural compound library used in this study was kindly provided by Korea Chemical Bank.

I also want to give my special thanks to the lab members, Ji Young Kim, Eun Jung Lee, and Yu Jeong Bae for their kind support and assisstances. I thank my colleagues in dermatology residency.

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ABSTRACT

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Hyperpigmented skin disorders such as melasma and lentigo are common photoaging diseases caused or exacerbated by ultraviolet (UV) radiation. A variety of molecules such as stem cell factor, endothelin-1 and alpha-melanocyte stimulating hormone have been identified as key mediators in the mechanisms of UV-induced melanogenesis. Preceding study from our group reported that adenosine 5'-triphosphate (ATP), which is a well-known molecular unit of intracellular energy, can increase melanogenesis through extracellular release of ATP in response to UVB. Thus extracellular ATP-P2X7 signaling axis was suggested to be an adjunctive mechanism in UV-mediated melanogenesis. Based on this background, this study tried to discover natural substances with anti-melanotic effect through inhibition of ATP-P2X7 axis. To discover potential chemical candidates affecting ATP-P2X7 pathway, cellular assay system was established through induction of P2X7 expression in human osteosarcoma cell line using lentivirus transduction and ATP-P2X7 signaling activity was detected with fluorescence of YO-PRO-1 dye. By high-throughput screening potential P2X7 inhibiting hit compounds were screened from natural compound library of Korea Chemical Bank. Among them, 7-desacetoxy-6,7-dehydrogedunin (7DG) was selected and was verified whether it reverses the melanogenic effect of ATP on primary human epidermal melanocytes (PHEM). Melanin content and the

expression level of melanogenesis related proteins such as tyrosinase, MITF, and gp100 increased when PHEM was incubated with 100 μM ATP. Melanin content and the expressions of melanogenesis-associated molecules increased by ATP were inhibited by 7DG treatment in a concentration-dependent manner.

This study suggests that 7DG could be a potential skin whitening material for the development of natural skin-lightening product.

Key words : melanogenesis, high-throughput screening, natural compound, ATP, P2X7, conessine, 7DG

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I. INTRODUCTION

Hyperpigmented skin disorders such as melasma and lentigo are common photoaging diseases caused or exacerbated by ultraviolet (UV) radiation. They most commonly occur on the face thereby causing cosmetic concerns. As interests in aesthetics and skin aging increase, and in particular, interest in whitening grows, the development of cosmetics and topical drugs for skin whitening is more active than ever.

Solar UVB radiation promotes melanin synthesis and distribution as well as increases melanocyte density[1]. The mechanism of UV-induced melanogenesis has been explored in many studies. Endothelin-1 and α-MSH have been identified as key mediators released from keratinocytes[2,3]. Their receptors, endothelin B and melanocortin-1 receptor are also activated in UV-induced melanogenesis. Tyrosinase, which is a key enzyme in mammalian melanogenesis increases during UVB-induced melanogenesis[4]. Therefore many compounds such as hydroquinone, arbutin, kojic acid, and 4-butyl resorcinol used in skin whitening products are targeting tyrosinase[5,6]. Nevertheless, there are many other molecules and pathways related to the mechanism of melanogenesis. However, there is still a lack of information about which is the most important and effective way to inhibit hyperpigmentation.

Adenosine 5'-triphosphate (ATP) is a well-known intracellular energy transferring molecule. However, ATP acts as an extracellular signal transducer through purinergic receptors under certain stress conditions such as inflammation, apoptosis, and hypoxia[7]. ATP and its metabolites are known to play their biologic roles via purinergic receptors. Purinergic receptors are largely divided into P1 and P2 receptors and P2 receptors are further subdivided into P2X ion channels and P2Y G protein-coupled receptors. Among 7 different subtypes of P2X receptor, P2X7 is previously known to be associated with infectious, inflammatory, autoimmune, neurological, and musculoskeletal disorders[8,9]. It has been proposed as a potential target for treating various inflammatory conditions[10]. In addition, P2X7 has also been found to play a critical role in UVB-induced melanogenesis from our group[11]. In that study, we found ATP is released from human keratinocytes and melanocytes after UVB irradiation. Extracellular ATP released by UV irradiation can induce melanin production and expression of melanogenesis -associated molecules in primary human epidermal melanocyte (PHEM).

For the following study, we found that a commercially available P2X7 inhibitor, AZD-9056, reduced ATP-induced melanin production [12]. Based on our previous studies, this study aimed to screen out possible P2X7 inhibiting hit compound from natural compound library by high-throughput screening and verify whether the select substances actually reduce ATP-induced melanogenesis.

II. MATERIALS AND METHODS

1. Cell line

Human osteosarcoma (HOS) cell line was used in the high-throughput screening. PHEM was isolated from human foreskin samples that were obtained after written informed consent in accordance with the study protocol approved by the Institutional Review Board of Severance Hospital (IRB no. 7-2017-1041). Cells were expanded in Dulbecco's Modified Eagle Medium (D-MEM) supplemented with 2% heat-inactivated fetal bovine serum and 1% penicillin/streptomycin (Sigma-Aldrich.,StLouis,MO).

2. Cytotoxicity assay

To determine cell viability, the [4,5-dimethylthiazol-2-yl]-2,5-diphenyltrazolium bromide (MTT) assay (Sigma-Aldrich) was used. All cells in a 96-well plate were incubated with MTT stock solution at (37℃ for 4 hours). After labeling with MTT, the cells were incubated for (10 minutes) in DMSO (Sigma-Aldrich) and then the absorbance of each sample was read at 570 nm.

3. Melanin content analysis

To assess melanin production, PHEM pellets were incubated in 1 N NaOH at 60℃ for 2 hours. Absorbance at 405 nm (detected with a plate reader) was compared to a synthetic melanin (Sigma-Aldrich) standard curve.

4. Tyrosinase activity analysis

Cells were lysed in radioimmunoprecipitation assay buffer. SDS sample buffer without mercaptoethanol was added to lysed samples. Samples were then resolved through10% SDS-PAGE. The gels were immersed in 0.1M NaH_2PO_4 (pH 6.8) for 30 minutes and then the buffer was replaced. After rinsing, buffer with 5 mM L-DOPA (SigmaAldrich) was added to the gels and incubated at 37℃for 3 hours. The darkened electrophoretic bands were then visualized.

5. Western blot analysis

Whole cell extracts were lysed in radioimmunoprecipitation assay buffer. Proteins were resolved by 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk in Tris-buffered saline and 0.1% Tween-20 and then incubated with primary antibodies against microphthalmia-associated transcription factor (MITF) (Abcam, Cambridge, UK), tyrosinase (Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A), gp100 (Abcam), and glyceraldehyde 3-phosphate dehydrogenase (Santa Cruz Biotechnology) at 4C overnight. Appropriate secondary antibodies were added and then the protein bands are detected using an enhanced chemiluminescence reagent.

6. High-throughput screening (HTS) of natural compounds with P2X7 inhibiting effect

To assay P2X7 activity, YO-PRO-1 was used[13]. The dye is fluorescent only when it bind nucleic acids. Thus the dye in culture medium is not fluorescent. When P2X7 is activated with high (millimolar) concentration of ATP, large-pore channel forms and enable YO-PRO-1 uptake followed by increased green fluorescence.

To establish HTS system for P2X7 assay, HOS-P2X7 cell stably overexpressing P2X7 was generated with lentiviral transduction in HOS cell line. The cells were plated on 96-well plate and grown to full confluency. The growth medium was replaced with HBSS containing 5 μM YO-PRO-1 and 25 μM each test compound, and then supplemented with ATP at a final concentration of 1 mM. Each assay plate contained no-ATP wells, vehicle-treated wells (negative control) and 25 μM A438079 (Sigma-Aldrich, one of the known P2X7 antagonists [14])-treated wells. After incubation at 37°C for 30 min, YO-PRO-1 fluorescence of each well was

read with exciation at 485 nm and emission at 520 nm. The difference in fluorescence of each well and mean of no-ATP wells was P2X7 acitivity. The percentage to negative control was considered as % P2X7 activitty. The % P2X7 inhibition was calculated as 100 - % P2X7 activity. The chemical library used in this study was kindly provided by Korea Chemical Bank (www.chembank.org) of Korea Research Institute of Chemical Technology.

7. Establishment of ATP-induced melanogenesis model with PHEM

MTT assay was performed to assess cell viability after pre-incubation of ATP at various concentrations (100 μM, 500 μM, 1mM, 3mM) and durations (day1, day3, day5). Along with the cell viability test, melanin concentration was measured. Appropriate ATP concentration, incubation time, and adequate cell density that shows increased melanin pigmentation was determined. α-MSH, a well-known substance that increases melanin synthesis was used as a positive control.

8. Verification of 7-Desacetoxy-6,7-dehydrogedunin (7DG) suppressing melanogenesis under ATP treatment

MTT assay was performed to assess cell viability after pre-incubation of 7DG at various concentrations (100 μM, 200 μM, 500 μM, 1mM, 2mM, 5mM, 10mM). Melanin content in 7DG and ATP-treated PHEM was compared with ATP-only-treated cells. Westernblot analysis was conducted to check protein levels of melanogenesis-related molecules, including MITF, tyrosinase, and gp100 after pre-incubation of 7DG on ATP-treated PHEM.

9. Statistical analysis

Data were analyzed with the unpaired Student's two-tailed t-test, unless otherwise stated, using the Prism software (GraphPad Software Inc). $P < 0.05$ was considered to be significant.

III. RESULTS

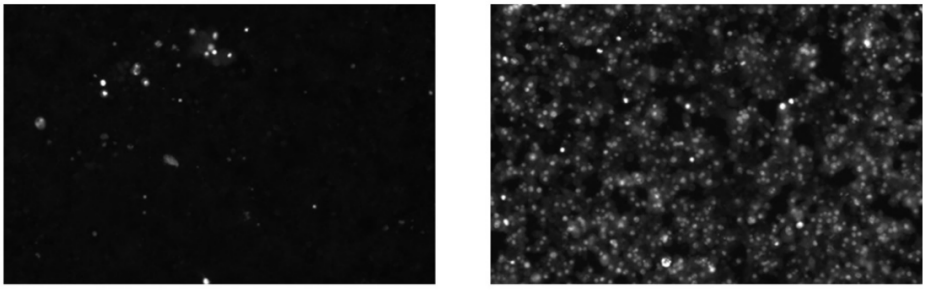
1. Extablishment of cell-based assay system to discover P2X7 antagonist

Cell-based assay system was established with HOS cell line overexpressing P2X7 via lentiviral transduction. The HOS-P2X7 cells were treated with 5 μM YO-PRO-1 for 30 minutes in the presence or absence of 25 μM of 1 mM ATP. The increase in green fluorescence was pronounced when P2X7 receptor was activated by 1 mM ATP treatmet (**Fig. 1A**). To find out optimal assay time the HOS-P2X7 cells grown on 96-well plate were treated with vehicle, 1 mM ATP, or 1 mM ATP combined with 25 uM A438079, and then with 5 μM YO-PRO-1. The green fluorescence of each well was measured at 5 min, 10 min and 30 min after the treatment. Z' factor is a statistical data quality indicator for HTS and its value over 0.5 is considered to have good separation quality[15]. Z' factor calculated from the result of 30-min ATP treatment was over 0.5, which suggest that the P2X7 assay system is adequate for HTS. (**Fig. 1B**).

2. HTS to identify natural substances with inhibitory effect on ATP-P2X7 pathway

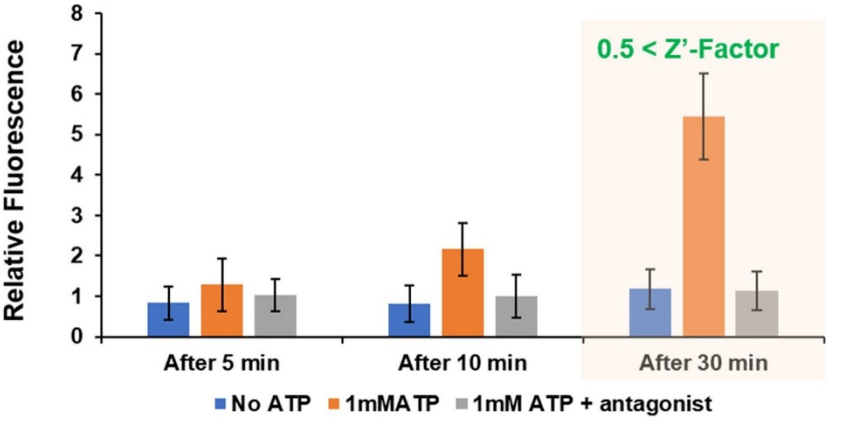
P2X7 HTS was performed to identify P2X7 inhibitors among 962 natural compounds provided by Korea Chemical Bank. Fiftey eight compounds showed more than 80% inhibition of P2X7 activity. Considering their cytotoxicity, 22 compounds were finally selected (**Fig. 1C**). P2X7 inhibition rate of these 22 compounds are shown in **Fig. 1D**.

A



No ATP　　1mM ATP

B



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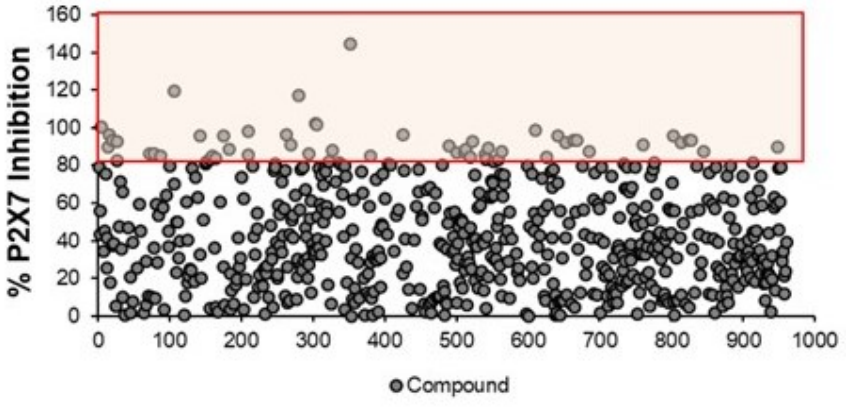

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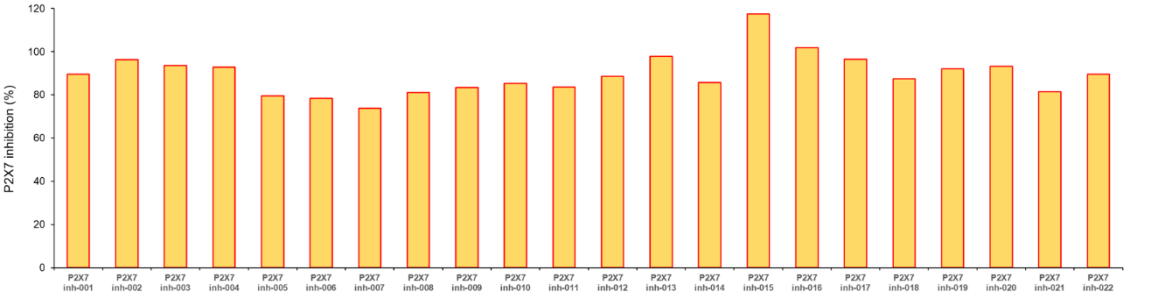

Figure 1. Identification of P2X7 inhibitors via HTS (A) HOS-P2X7 cells were treated with YO-PRO-1 alone or combined with 1 mM ATP to activate P2X7. After 30 min of incubation, fluorescence images were obtained via GFP filter and presented. (B) HOS-P2X7 cells grown on 96-well plate were treated with YO-PRO-1 in the presence of vehicle, 1 mM ATP, or 1 mM ATP combined with a P2X7 antagonist, A438079. Green fluorescence was measured at 5 min, 10 min and 30 min after incubation and presented as bar graphs. Z' factor was calculated from the results. (C) HTS result of 962 natural compounds from Korea Chemical Bank. Compounds with more than 80% inhibition were selected as initial hits and shown in a red box. (D) Strong inhibition of P2X7 was confirmed in 22 selected compounds. (The results in this figure were obtained from Dr. Eun Ju Choi)

Abbreviations : ATP, adenosine 5'-triphosphate

3. ATP induces the expression of melanogenesis related proteins and melanin contents

MTT assays of ATP treated PHEM revealed that cell viability was not affected at 100 μM and 500 μM ATP treatment but significantly reduced at 1mM ATP treatment (**Fig. 2A**).

At the adequate concentration which does not affect cell viability, ATP treatment in PHEM induced melanogenesis. As shown in **Fig. 2B**, melanin content increased at day 3 of 100 μM ATP treatment. Consistently western blot analysis revealed that MITF and tyrosinase expression level increase in ATP treated PHEM (**Fig. 2C**). To summarize the data, ATP can threaten cell survival at high dose, but at the concentration under 500 μM, ATP promotes melanin synthesis in PHEM.

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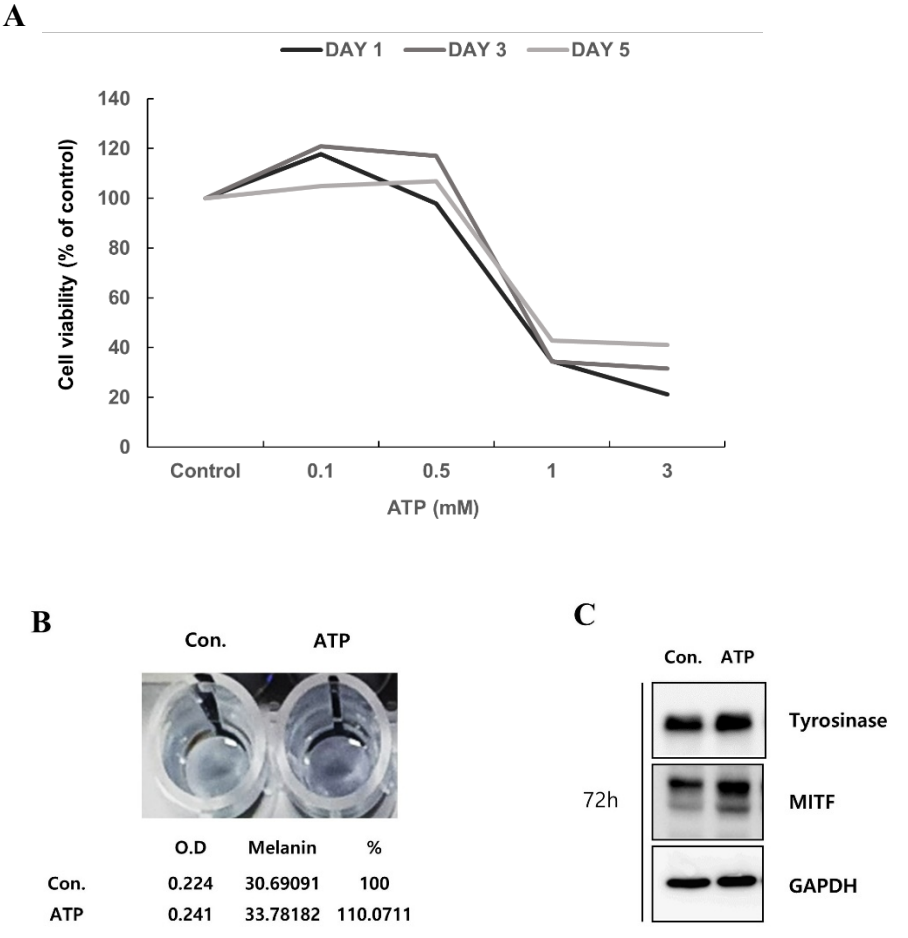


Figure 2. (A) MTT assay of PHEM incubated with various concentrations of ATP. Cell viability was maintained up to 500 μM of ATP incubation but drastically reduced at 1mM ATP treatment. (B) Melanin contents were increased at day 3 of 100 μM ATP treatment in PHEM. (C) Expression level of melanogenesis related proteins, MITF and tyrosinase increased when 100 μM of ATP was treated in PHEM.

Abbreviations : ATP, adenosine 5'-triphosphate; *MITF,* microphthalmia-associated transcription factor; *PHEM,* primary human epidermal melanocyte

4. Inhibition of P2X7 receptor suppresses ATP mediated melanogenesis in PHEM

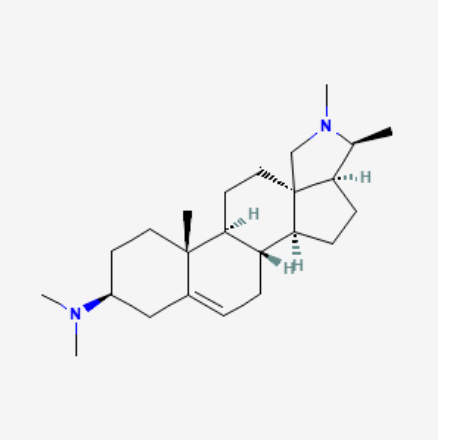
Among several hit compounds in the natural compounds chemical library, which have more than 80% of inhibition effect on ATP-P2X7 system through high-throughput screening, conessine and 7-dexacetoxy-6,7-dehydrogedunin (7DG) were selected to verify whether it has also inhibiting effects on ATP-P2X7-mediated melanogenesis. Conessine is a steroidal alkaloid and histamine H3 receptor antagonist[16] and is isolated from *Holarrhena floribunda*. 7DG with the molecular weigth of 422.5g/mol is structurally similar to gedunin (**Fig. 3A, B**), a heat shock protein 90 (Hsp90) inhibitor and is known to bind protein kinase R (PKR) outside the ATP catalytic domain thereby inhibiting PKR activity[17]. Cell viability of PHEM according to different concentrations of 7DG and conessine is shown in **Fig. 4A and B**. Less than 5 μM of 7DG did not affect melanocyte survival , but 10 μM of 7DG decreased cell viability. Cell viability was maintained until 2 μM of conessine incubation but started to decrease by from treatment of 5 μM of conessine.

Based on this result, 0.5, 1, and 2 μM of 7DG and conessine were co-treated with 100 μM ATP in PHEM. On 1 μM and 2 μM of 7DG suppressed tyrosinase, gp100, and MITF expression level that were increased by ATP treatment (**Fig. 4C, D**). Tyrosinase activity, which was increased by ATP incubation was also reduced by 7DG treatment (**Fig. 4E**). The expression level of tyrosinase increased when 100 μM of ATP was treated in PHEMs but decreased when co-treated with 0.5 μM, 1 μM and 2 μM (**Fig. 4F**). 7DG and conessine decreased melanin content, a final product of melanogenesis in ATP-treated PHEM **(Fig. 4G and H)**. Although conessine has demonstrated its antimelanogenic effect on ATP induced hyperpigmented melanocytes, further experiment was held because this compound is currently patented in the U.S. for its use as antimelanogenic and pigment modulating compound.

Interestingly when 7DG was treated in PHEMs alone, gp100 and tyrosinase

expression level was significantely reduced at 1 μM and 2 μM concentration (**Fig. 5A, B**). Tyrosinase activity also decreased in 1 μM and 2 μM 7DG treated PHEMs (**Fig. 5A, C**). As shown in **Fig. 5D**, melanin content assessed by 405nm absorbance significantly decreased in 1 μM and 2 μM 7DG treated PHEMs.

A



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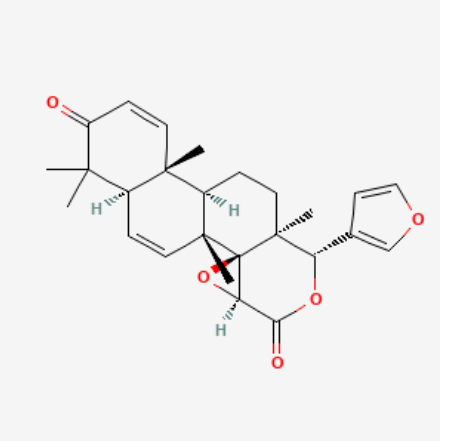
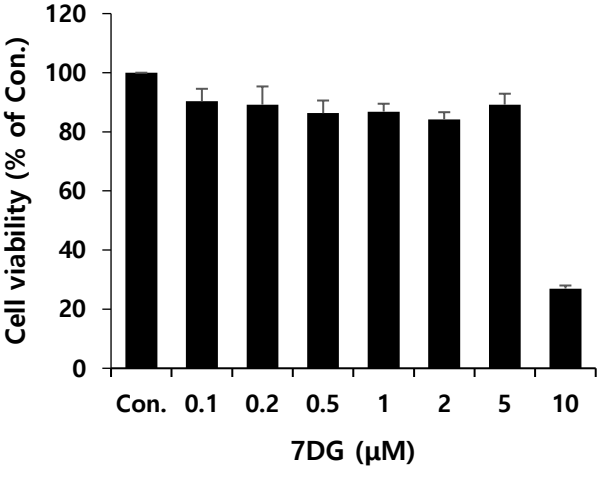


Figure 3. Chemical structure of (A) conessine and (B) 7-dexacetoxy-6,7-dehydrogedunin (7DG). The chemical formula of conessine is $C_{24}H_{40}N_2$ and the molecular weight is 256.6g/mol. The chemical formula of 7DG is $C_{26}H_{30}O_5$ and the molecular weigt is 422.5g/mol.

A



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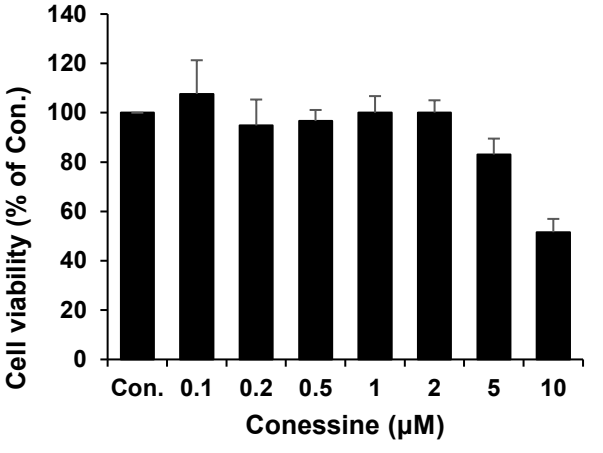

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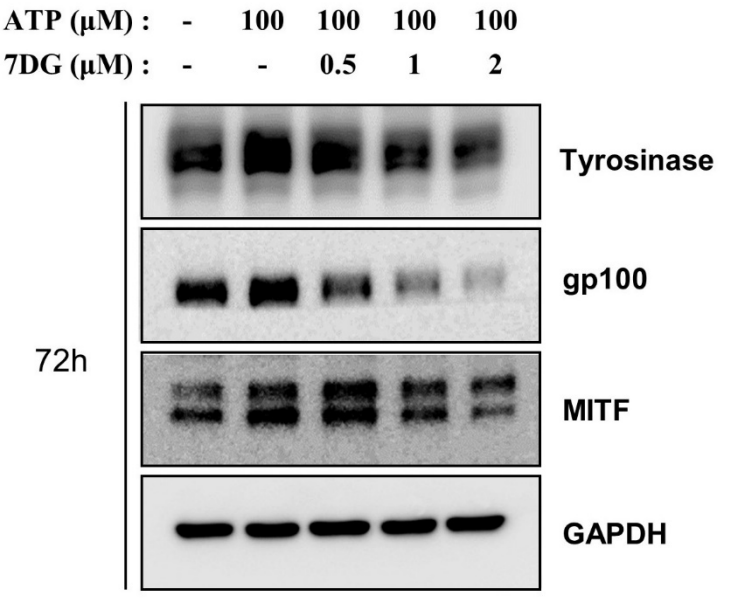

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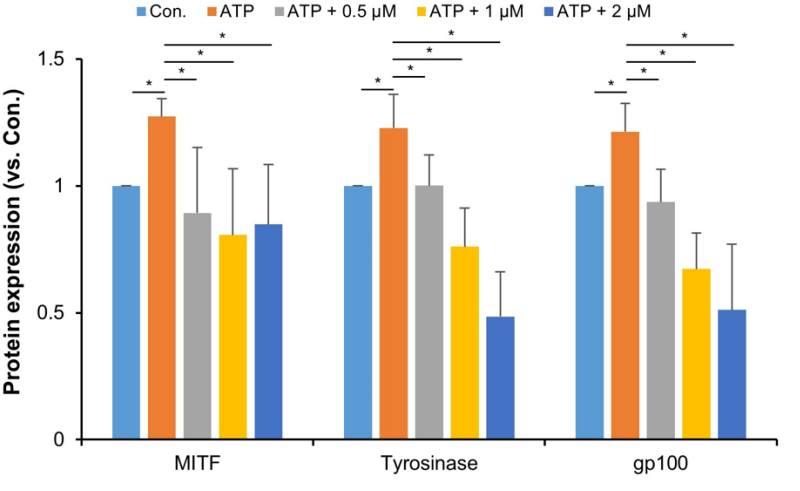

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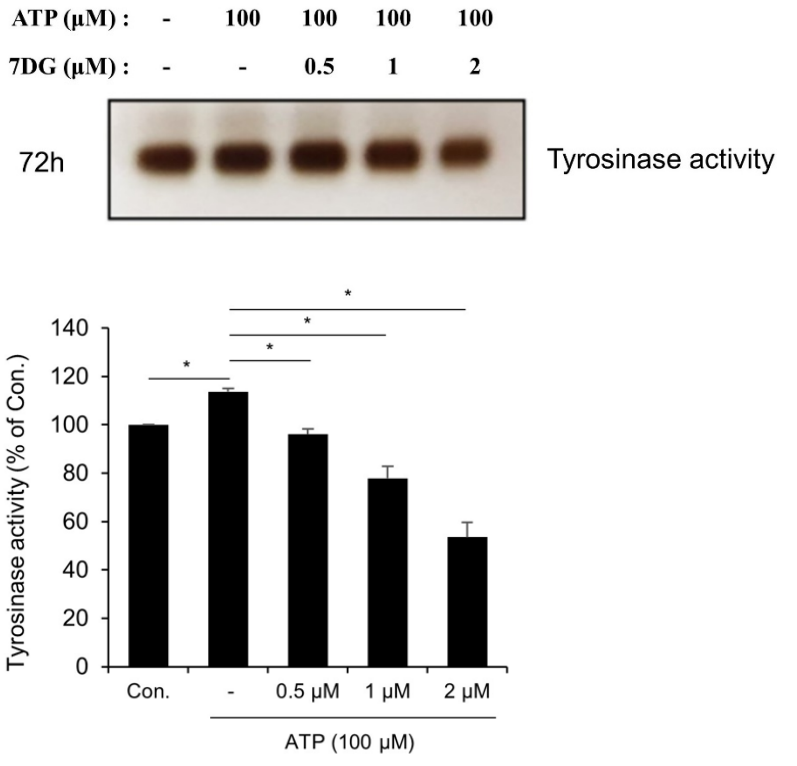

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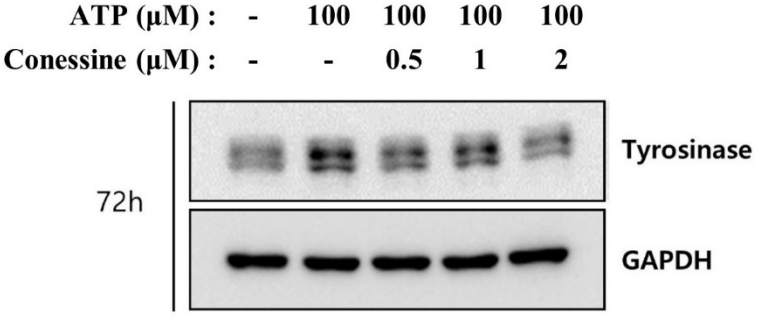

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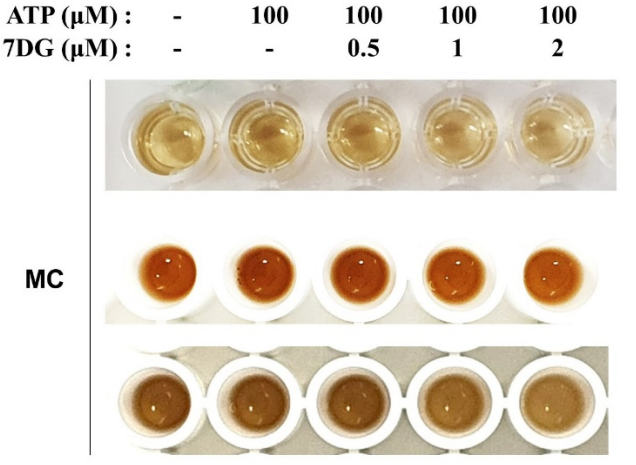

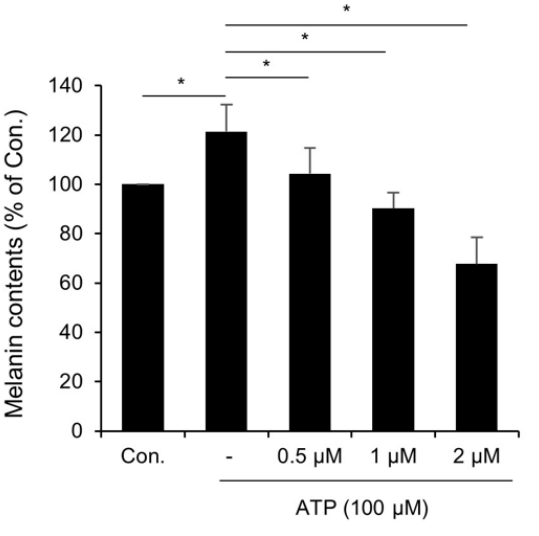

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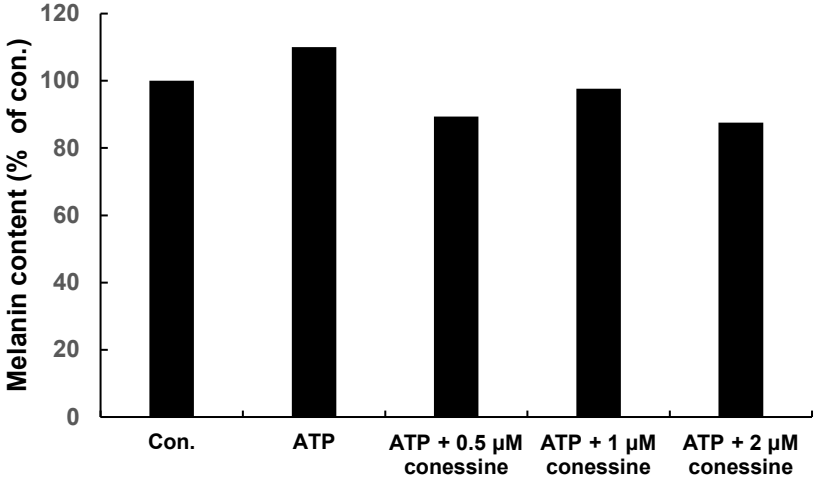
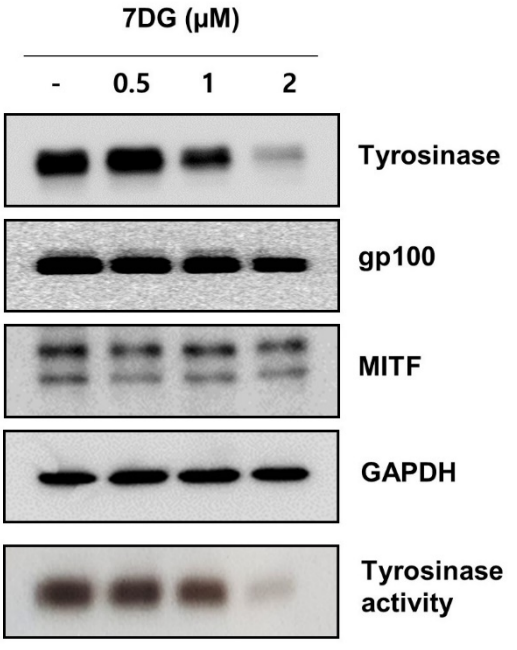


Figure 4. (A, B) MTT assay of PHEMs treated with various concentrations of 7DG and conessine. (C, D) Expression level of tyrosinase, gp100, and MITF increased when 100 μM of ATP was treated in PHEMs but was suppressed when cotreated with 0.5 μM, 1 μM and 2 μM 7DG. (E) Tyrosinase activity was increased when PHEM was incubated with 100 μM ATP and reduced in 2 μM 7DG cotreated PHEM. (F) Expression level of tyrosinase increased when 100 μM of ATP was treated in PHEMs but was suppressed when cotreated with 0.5 μM, 1μM and 2μM conessine. (G) PHEM treated with ATP and 7DG. Melanin content increase was suppressed when 7DG was admixed with ATP. (H) PHEM treated with ATP showed increased melanin content but this increase was reversed when co-treated with 0.5μM, 1μM, and 2μM conessine.

Abbreviations : 7DG, 7-desacetoxy-6,7-dehydrogedunin; *ATP*, adenosine 5'-triphosphate; *MITF,* microphthalmia-associated transcription factor; *PHEM,* primary human epidermal melanocyte

A



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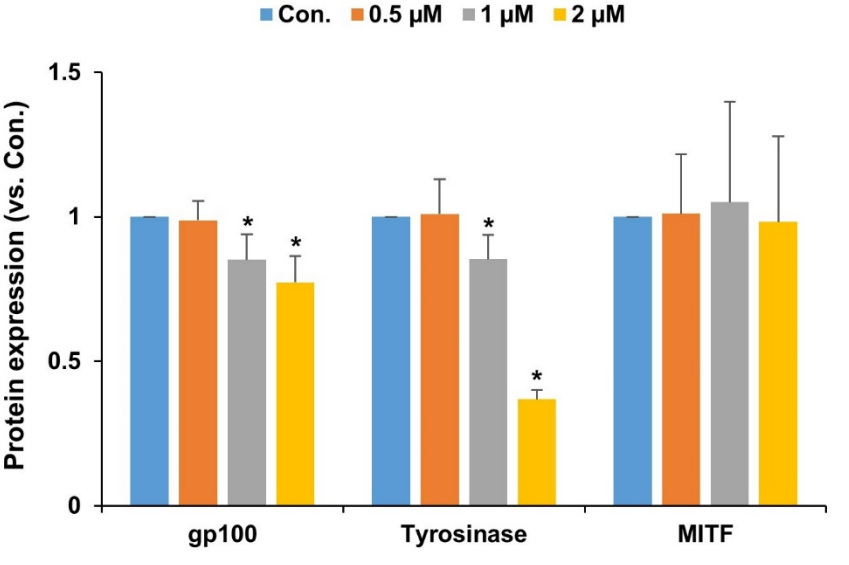

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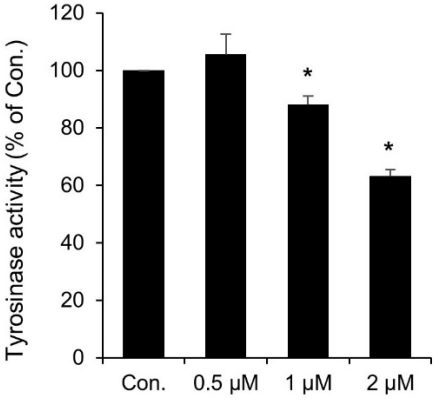

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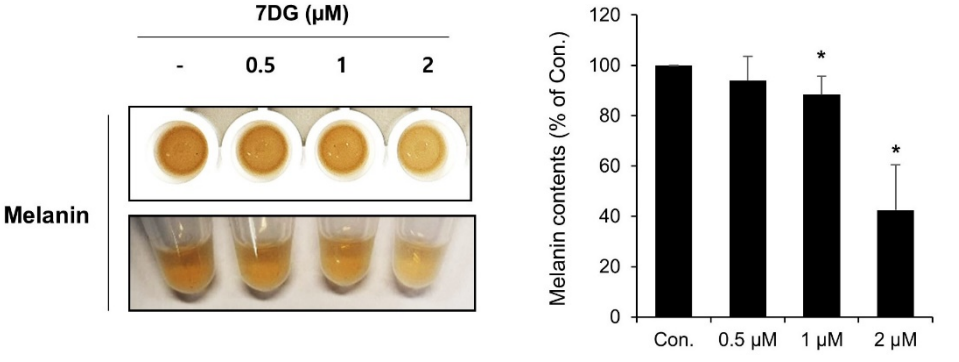

Figure 5. (A, B) Expression level of gp100 and tyrosinase significantly decreased when 1 and 2 μM 7DG was treated in PHEMs. (C) Tyrosinase activity was also significantly reduced when 1 and 2 μM 7DG was treated in PHEMs. (D) PHEM treated with 7DG showed decreased melanin contents at 1 and 2 μM concentration of 7DG.

Abbreviations : 7DG, 7-desacetoxy-6,7-dehydrogedunin;*MITF,* microphthalmia-associated transcription factor; *PHEM,* primary human epidermal melanocyte

IV. DISCUSSION

Extracellular ATP in human skin acts as a direct signaling molecule that triggers inflammatory, regenerative, and fibrotic responses to mechanical injury[18]. It also indirectly regulates melanocyte proliferation and apoptosis. ATP mediated melanogenesis is reported to have a role in UV-induced melanin synthesis[11]. Previous work from our group demonstrated that UVB irradiation renders primary human epidermal keratinocytes and PHEMs to release ATP. It was speculated that extracellular ATP released from keratinocytes and melanocytes may trigger melanin synthesis in epidermal melanocytes. Furthermore, well known P2X7 inhibitors, AZD-9056 and A438079 are known to reduce ATP-induced melanin synthesis[12]. Therefore, targeting ATP can be an effective way to suppress ATP mediated melanogenesis, and even UVB-associated melanin synthesis. Unlike AZD-9056 and A438079, 7DG, which was found from high throughput screening system in this study is distinct as it is a naturally occurring single compound.

Hight throughput screening has been used to discover antimelanogenic compound in the past[19-21]. Previous studies directly checked pigment or the expression level or activity of melanin synthesis related proteins thus used melanoma cells or immortalized melanocytes. In this study, P2X7 receptor was set as a target before screening natural compounds in the chemical library. Therefore we employed cellular assay system used in high-throughput screening for discovering P2X7 inhibitors. A previous study that identified P2X receptor inhibitors by screening animal venoms used the assay system similar to our study with calcium-sensitive fluorescent dye and dye uptake probe YO-PRO-1[13]. Another study which found P2Y6 inhibitor used intracellular Ca^{2+} concentration as an indicator for P2Y6 activity[22]. Both studies used human astrocytoma cell lines (1321N1 cells) with or without human embryonic kidney cells (HEK293T cell line). Our group initially set up cell-based assay system with HEK293T cells, but switched to HOS cell because of the weak adhesion property of HEK293T

cells.

7DG is structurally similar to gedunin, a limonoid natural compound extracted from plants of the Meliaceae family and especially abundant in the Indian neem tree (Azadirachta indica)[23]. Biological activities of gedunin are well attributed to antibacterial, antimalarial, and insecticidal[23,24]. Gedunin is also known to disrupt Hsp90 and is responsible for maintaining cellular homeostasis theryby induce apoptosis in lung cancer and exert anti-proliferative activity in ovarian cancer cells[25,26]. Recently antimelanogenic effect of gedunin and other limonoids extracted from Azadirachta indica has been identified in murine melanoma cells and in animal models[27,28].

In this study, the initial plan of this study aimed to screen out ATP-P2X7 inhibiting molecules by high throughput screening and the final goal was to discover single natural compound with anti-melanogenic effect via ATP-P2X7 pathway. Therefore, it needed verification regarding whether 7DG, which was selected to have an ATP-P2X7 inhibiting effect can suppress ATP-mediated melanogenesis in PHEMs. As a result, the melanin content and melanogenesis related protein expression levels, which were increased by ATP treatment are effectively reduced by 7DG. Therefore, antimelanogenic effect of 7DG is thought to be mediated through ATP-P2X7 pathway.

Interestingly, compared to untreated control melanocytes, the expression levels of tyrosinase, MITF, and gp100 were much lower in PHEMs treated with 2 μM of 7DG (high concentration) without affecting cell viability even if ATP as a melanogenic molecule was added. Moreover, 1 μM 7DG alone without ATP treatment reduced gp100 and tyrosinase expression and showed whitening effects in PHEMs. This could be because 7DG interfered with the normal melanogenesis process by further suppressing baseline P2X7 activity. Another explanation is that 7DG not only reverses ATP-mediated melanogenesis in its low concentration but also can act as a further powerful hypopigmenting in high concentration agent through unknown other mechanisms. Further study is needed to elucidate the

mechanism how high concentration of 7DG inhibits melanogenesis aside from ATP-P2X7 pathway. In addition, the appropriate concentration of 7DG that effectively inhibits pigment formation without remarkable side effect should be investigated through *in vivo* clinical studies.

UV radiation causes DNA damage on skin cells, and melanin acts to protect against it. It is known that dark pigmented skin is more protective of photocarcinogenesis and photoaging[29]. However excessive pigmentation such as lentigo and melasma is a major cosmetic problem. Thus skin whitening agents are at high demands especially in Asian population. Currently topical skin whitening agents mostly target tyrosinase, a key enzyme for melanin synthesis. A lot of natural substances targeting tyrosinase have been discovered to have anti-melanotic effect[30]. However, several concerning issues were raised in the use of tyrosinase inhibitors for bleaching purpose. Hydroquinone, a prototype of tyrosinase inhibitor has been connected with mutagenicity and is now banned by the EU cosmetic regulation. A naturally occurring tyrosinase inhibitor, rhododendrol was once commercialized in Japan as skin-whitening cosmetics, but it was reported to have induced vitiligo and chemical leukoderma in a mass population. After 5 years on the market, rhododenrol is now withdrawn. It has been revealed that rhododenrol is oxidized to rhododendrol quinones by tyrosinase, and thus obtain cytotoxic effects against melanocytes[31]. Therefore the need to discover another target for skin whitening agents has emerged. Considering that melanin production is regulated by multiple enzymes and molecules, combined approaches could be better for the development of anti-melanotic agents in the aspect of side effects. Tageting ATP-P2X7 axis can be a smart alternative way to control excessive melanin synthesis, because it not only regulates melanogenesis but also is related to inflammation in damaged cells. Prolonged inflammation in the skin is well known to cause pigmentation. This condition called postinflammatory hyperpigmentation is a major indication for topical skin whitening products. As inhibiting P2X7 receptors can control

inflammation, it could improve skin environment and have synergistic effect in suppressing excessive pigmentation.

In conclusion, 7DG, a natural P2X7 inhibitor could be a promising single natural compound for anti-melanotic effect.

V. CONCLUSION

By means of high-throughput screening, natural substances that have P2X7 inhibiting property were discovered. Among them, 7DG was found to effectively suppress ATP-induced melanogenesis without affecting melanocyte viability. This study could be the basis for development of natural skin whitening agents.

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ABSTRACT(IN KOREAN)

ATP-P2X7 axis를 통한 색소형성 억제 천연물 유래 물질의 발굴

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박 수 진

기미, 흑자 등 과색소질환은 자외선에 의해 유발 혹은 악화되는 흔한 광노화 질환으로 얼굴에 발생하여 미용적인 문제를 야기한다. 줄기세포인자, endothelin-1, α-MSH와 같은 다양한 물질이 자외선에 의한 멜라닌 생성 기전의 핵심 매개체로 알려져 있다. 현재까지 색소질환에 이용되는 외용제의 기전은 멜라닌색소 합성의 주요 효소인 tyrosinase를 억제하는 것이 가장 보편적이나 tyrosinase 억제제로 개발된 물질은 피부에 자극 반응이 있을 뿐 아니라 안전성에 관한 우려도 있는 실정이다.

Adenosine 5'-triphosphate(ATP)는 세포내 에너지 전달물질로서 그 기능이 잘 알려져 있지만 염증, 세포사멸, 저산소 등 특정 조건 하에서 퓨린수용체를 통해 세포 밖 신호전달자 역할도 한다. 본 연구에 앞선 선행연구를 통해 자외선에 의해 각질형성세포로부터 유리된 ATP가 멜라닌세포의 P2X7 수용체에 작용하여 멜라닌 합성을 유도하는 것을 확인한 바 있다. 이번 연구에서는 P2X7을 억제할 수 있는 천연물 hit compound를 high throughput screeening system을 통해 발굴하고 이 물질이 실제 ATP를 통한 멜라닌 합성을 억제하는지 검증하였다. P2X7 수용체 발현을 유도한 인간 골육종 세포주에서 P2X7 수용체를 통과하여 세포내로 이동하는 형광 염료를 사용하여 P2X7 활성도를 측정하는 분석 시스템을 정립한 후

한국화합물은행을 통해 제공받은 962종의 천연물을 선별한 결과 ATP에 의해 증가된 멜라닌색소 형성을 억제하고 멜라닌색소 형성에 관여하는 주요 효소의 발현을 억제하는 7-desacetoxy-6,7-dehydrogedunin (7DG)을 발굴하였다.

본 연구가 향후 천연물 유래 물질인 7DG를 원료로 한 피부 미백제 개발로 이어질 수 있을 것으로 기대한다.

핵심되는 말 : 멜라닌색소, 천연물, high-throughput screening, ATP, P2X7, conessine, 7DG