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Identification of new arylsulfide derivatives as anti-melanogenic agents in a zebrafish model

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ABSTRACT

A series of aryl sulfide derivatives was synthesized and evaluated for their anti-melanogenic activities. Several compounds, including **3e**, **3i** and **3q** exhibited good anti-melanogenic activities. Among the derivatives, compound **3i** showed good inhibitory effects against melanin synthesis and showed no toxicity in reconstituted human eye and skin tissues.

Melanin is a cluster of dark polymer pigments responsible for eye, hair, and skin color and these pigments are commonly present in almost all animals.[1] Melanin is synthesized by melanocytes in the basal layer of the epidermis via a process called melanogenesis.[2,3] Complex enzymatic and signaling pathways are involved in melanin synthesis. Tyrosinase (TYR), tyrosinase-related protein-1 (TRP-1), and tyrosinase-related protein-2 (TRP-2) are three key regulators of melanin synthesis. Melanogenesis is mediated by a series of complex signaling pathways initiated by internal and external factors, including ultraviolet radiation (UVR), nitric oxide (NO), and stress.[1] Each of the signaling pathways is associated with a master regulator called MITF, which controls the expression of genes such as TYR, TRP-1, and TRP-2.

Normal melanin pigmentation has several beneficial effects, including protection of skin cells from damage from UV light, toxic drugs, and other chemicals.[4,5] In contrast, abnormal production of melanin causes acute dermatological problems, including senile lentigines, freckles, melasma, post-inflammatory hyperpigmentation, and age spots.[6–8] These dermatological problems can lead to severe emotional issues; therefore, the development of anti-melanogenic agents is necessary.

The most common commercially available cosmetics or skin whitening agents such as hydroquinone[9,10], kojic acid[11], and arbutin are tyrosinase inhibitors.[12] Although they are recommended worldwide, they are associated with certain drawbacks and side effects. Hydroquinone is toxic to mammalian cells[13,14] and associated with a series of side effects.[15] The usage of kojic acid has been restricted due to its carcinogenicity and instability, and arbutin shows reduced efficacy in vivo.[16,17] Thus, there is a keen interest in the identification of new anti-melanogenic compounds.

In this study, to identify anti-melanogenic agents, we screened ~1000 compounds in the Korea Chemical Bank library by phenotype screening using live zebrafish. We positively identified compound **KDZ-003** as a hit. It exhibited moderate anti-melanogenic activity in the zebrafish model. The initial outcome encouraged us to perform scaffold modification. We wish to report the synthesis and biological evaluation of arylsulfide derivatives as anti-melanogenic agents

A series of sulfide derivatives were synthesized according to Scheme 1. Commercially available 1-chloroisoquionoline **(1a)** reacted with diverse thiols **(2)** such as benzothiazole, imidazole, pyridine, pyrimidine, and phenyl in ethanol to provide 1-thio-isoquinoline derivatives **(3a~q)**. Subsequently, not only 1-chloroisoquinoline, but also purine, pyrrolo[2.3-*d*]pyrimidine, pyrrolo[3.2-*d*]pyrimidine, and thieno[3,2-*d*] pyrimidine **(1b-e)** were coupled with 4-fluorophenyl thiols **(2a)** to obtain sulfide compounds **(3n~q)**. Compounds **4a** and **4b** were synthesized by the treatment of 1-chloroisoquinoline **(1a)** with 4-fluorophenol **(2b)** and 4-fluoroaniline **(2c)** as depicted in Scheme 2

In our phenotypic screening assay, we screened ~ 1000 compounds to identify anti-melanogenic agents. After treatment for 24 h (between 10 and 34 hpf), **KDZ-003** affected melanin synthesis with no developmental defects. In an effort to increase its anti-melanogenic activity, **KDZ-003** was optimized, and the results were summarized in Tables 1–4. Compound **3a** showed better anti-melanogenic efficacy than **KDZ-**

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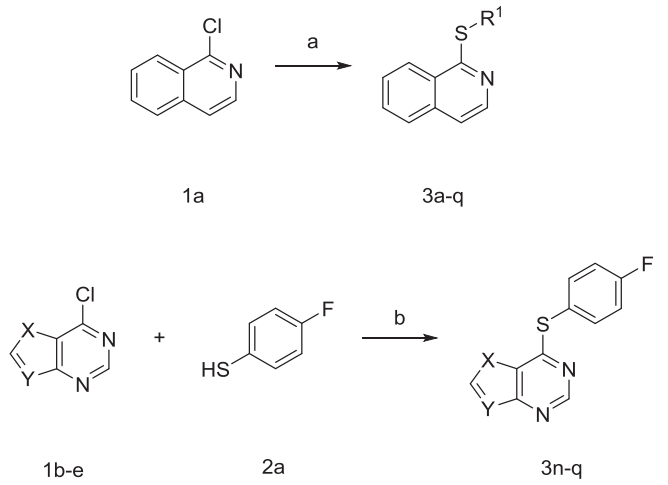
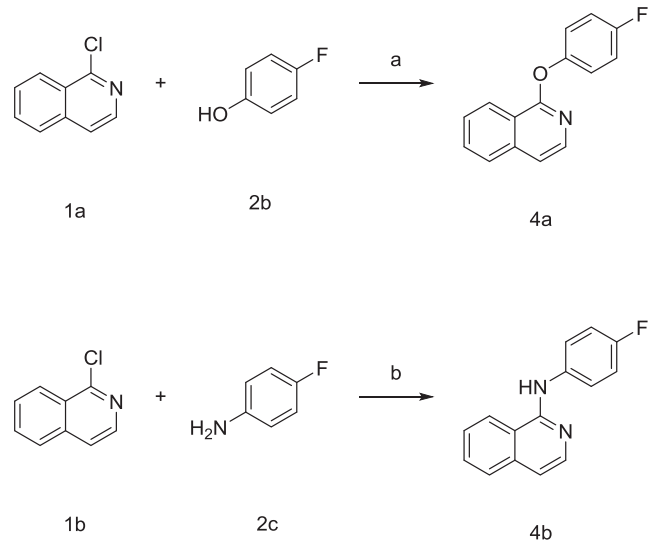


Scheme 1. Reagents and condition: a) R^1SH, EtOH, room temperature, 16 h; b) trimethylamine, 1-butanol, reflux, 16 h; *Note*: Substituents (R^1, X and Y) are indicated in Tables 1-4.

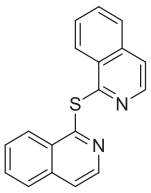
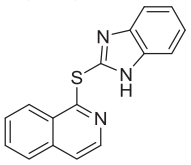
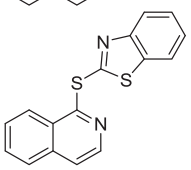
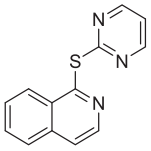
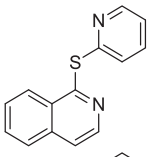
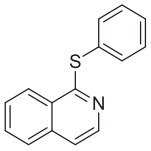


Scheme 2. Reagents and condition: a) CuI, K_2CO_3, DMF, 150 °C, 6 h; b) HCl, EtOH, 90 °C, 8 h.

003, however, compound **3b**, which has benzothiazole, was not active. Therefore, we sought monocyclic aromatic moiety. Pyrimidine **(3c)**, pyridine **(3d)**, phenyl derivatives **(3e)** were synthesized and evaluated. Compound **3c** showed lesser anti-melanogenic effect than **3a**. Compound **3d** and **3e** exhibited good anti-melanogenic effect. However, compound **3d** caused cell death. Therefore, we further modified phenyl derivative with diverse substituents.

Diverse phenyl derivatives were synthesized and the results are summarized in Table 2. The substituents showed increased in vitro activity, especially those with halide groups **(3h, 3i)** unlike compounds with ethyl ester **(3l)** and trifluoromethyl **(3m)** groups. Among the halide derivatives, the 4-fluoro compound **(3i)** showed the most potent

Table 1
Anti-melanogenic efficacy of compounds **3a-e** against zebrafish embryos.

No	Compound	Structure	Whitening efficacy [a] test based on zebrafish embryos (10 μM)
1	**KDZ-003**		+
2	**3a**		+ +
3	**3b**		–
4	**3c**		+
5	**3d**		+ +
6	**3e**		+ +

[a] Excellent (+ + +), Good (+ +), Moderate (+), No (-).

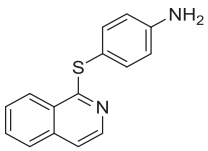
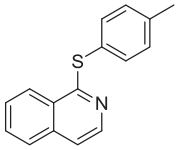
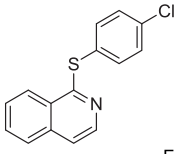
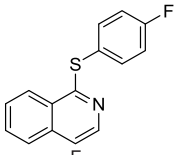
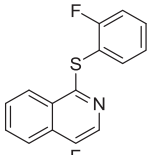
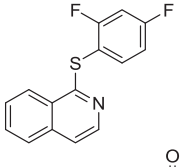
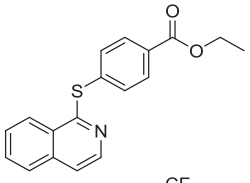
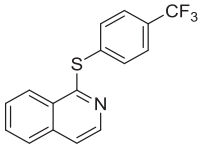
whitening efficacy. We synthesized more fluorine-containing compounds, via addition **(3j)** or repositioning **(3k)**, and found that the mono 4-fluoro derivative is more favorable for in vitro activity.

In addition, we tried to expand the sulfide into ether and amine derivatives. Interestingly, when the thioether group is changed into the ether **(4a)** and amine **(4b)**, the anti-melanogenic effect disappeared; indicating that the sulfide group is essential for the anti-melanogenic efficacy.

Based on these results, we fixed the 4-fluorophenyl sulfide group and changed the 1-isoquinoline group into different kinds of fused heterocycles. As shown in Table 4, modification to purine **(3n)**, pyrrolo[2.3-*d*]pyrimidine **(3o)** and, pyrrolo[3.2-*d*]pyrimidine **(3p)** decreased the anti-melanogenic effect compared to that of **3i**. Whereas, thieno[3,2-*d*]pyrimidine **(3q)** showed potent anti-melanogenic effect.

From the zebrafish screening results, we chose active compounds

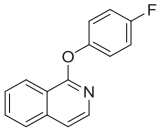
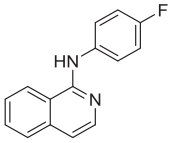
Table 2
Anti-melanogenic efficacy of compounds **3f-m** against zebrafish embryos.

No	Compound	Structure	Whitening efficacy [a] test based on zebrafish embryos (10 μM)
1	**3f**		+ +
2	**3g**		+ +
3	**3h**		+ +
4	**3i**		+ + +
5	**3j**		+ +
6	**3k**		+ +
7	**3l**		+
8	**3m**		–

[a] Excellent (+ + +), Good (+ +), Moderate (+), No (−).

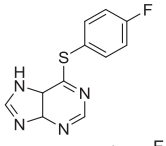
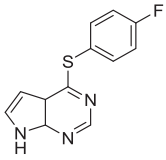
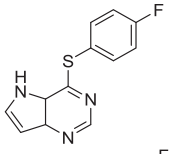
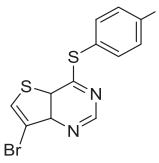
(**3e**, **3i** and **3q**) for a dose-dependency study using the zebrafish model. We confirmed that **3i**, **3q**, and **3e** treatment in zebrafish embryos resulted in anti-melanogenic effects in a dose-dependent manner (Fig. 1C-K) compared to DMSO, and PTU-treated control embryos (Fig. 1A, B).

Table 3
Anti-melanogenic efficacy of compounds **4a-b** against zebrafish embryos.

No	Compound	Structure	Whitening efficacy [a] test based on zebrafish embryos (10 μM)
1	**4a**		–
2	**4b**		–

[a] Excellent (+ + +), Good (+ +), Moderate (+), No (−).

Table 4
Anti-melanogenic efficacy of compounds **3n-q** against zebrafish embryos.

No	Compound	Structure	Whitening efficacy [a] test based on zebrafish embryos (10 μM)
1	**3n**		–
2	**3o**		+
3	**3p**		+ +
4	**3q**		+ + +

[a] Excellent (+ + +), Good (+ +), Moderate (+), No (−).

Based on the anti-melanogenic effects (Fig 1) as well as the side effects including edema (data not shown), we chose compound **3i** as a prototype. The effect of **3i** on melanogenesis-related proteins and genes level in the HMV-II cell lines was investigated. We examined the color in the pellets after **3i** treatment, in which the melanin changed from black to brown (Fig 2A). Treatment of **3i** reduced melanin contents in a dose-dependent manner. HMV-II cell lysates showed 67.6%, 31.3%, and 14.6% of the melanin contents of the DMSO control after treatment

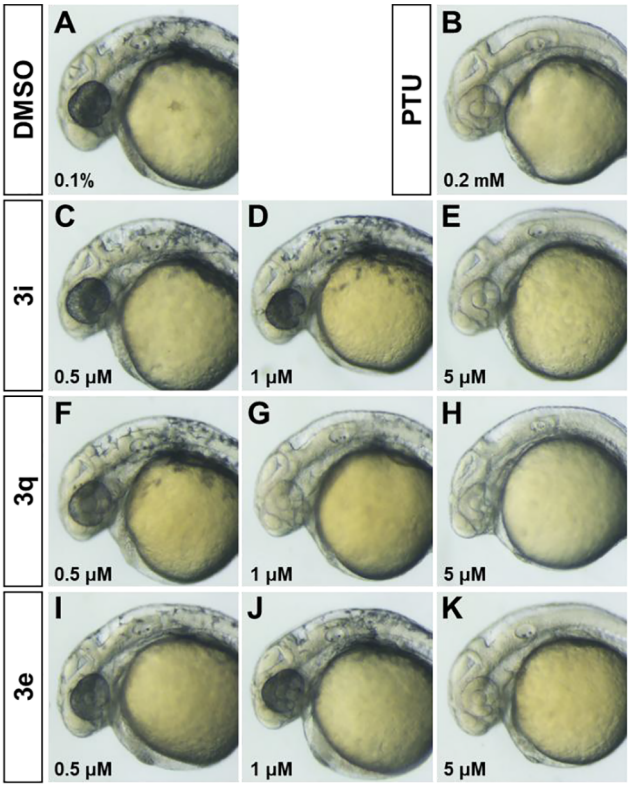


Fig. 1. Assessment of anti-melanogenic effect in zebrafish embryos. Synchronized embryos were treated with (A) 0.1% DMSO and (B) 0.2 mM PTU. (C-E) **3i**, (F-H) **3q**, and (I-K) **3e** treatments exhibited anti-melanogenic effects in a dose-dependent manner.

with 1, 10, and 20 μM 3i, respectively (Fig. 2B). Compound **3i** more potently decreased the melanin content than that of arbutin and kojic acid, which were used as a positive control (Fig. 3).

The effect of **3i** on the expression of melanogenesis-related genes was assessed by real-time RT-PCR. As shown in Fig 2C, 3i treatment markedly inhibited mRNA transcriptional levels of TRP-1, TRP-2, and MITF in a dose-dependent manner in HMV-II cells. Although the level of tyrosinase mRNA was also reduced by treatment of 10 or 20 μM 3i, howerver was not as clear as other genes and there was no concentration-dependent decrease. These findings demonstrate that the inhibitory effects of **3i** on melanogenesis in HMV-II cells might be mediated through the downregulation of melanogenic genes TRP-1, TRP-2, and MITF.

Cytotoxic effects of **3i** on HMV-II cells were measured with CytoX^{TM} solution. When the HMV-II cells were treated with 20 μM **3i,** the decrease in cell survival rates was less than 10%. This result suggests that **3i** is less cytotoxic and safer in the range of the whitening effect. (Fig 3G)

The safety of 3i was tested in artificial ocular and skin tissue, Neoderm-CD and Neoderm-ED, respectively. We determined the viability of artificial tissues in the presence of **3i** using an MTT assay. As a result, **3i** showed no toxic effects up to 50 μM in both Neoderm-CD (Fig. 4A, B) and Neoderm-ED (Fig. 4C, D), which would indicate that **3i** is safe for general cytotoxicity.

In summary, a new series of aryl sulfide derivatives was synthesized and evaluated for their anti-melanogenic effect using zebrafish and mammalian cells. Here, compounds **3e**, **3i** and **3q** showed significant anti-melanogenic effect in the zebrafish model. Among the derivatives, compound **3i** showed good inhibition of melanin contents and no toxicity with reconstituted human eye and skin tissue.

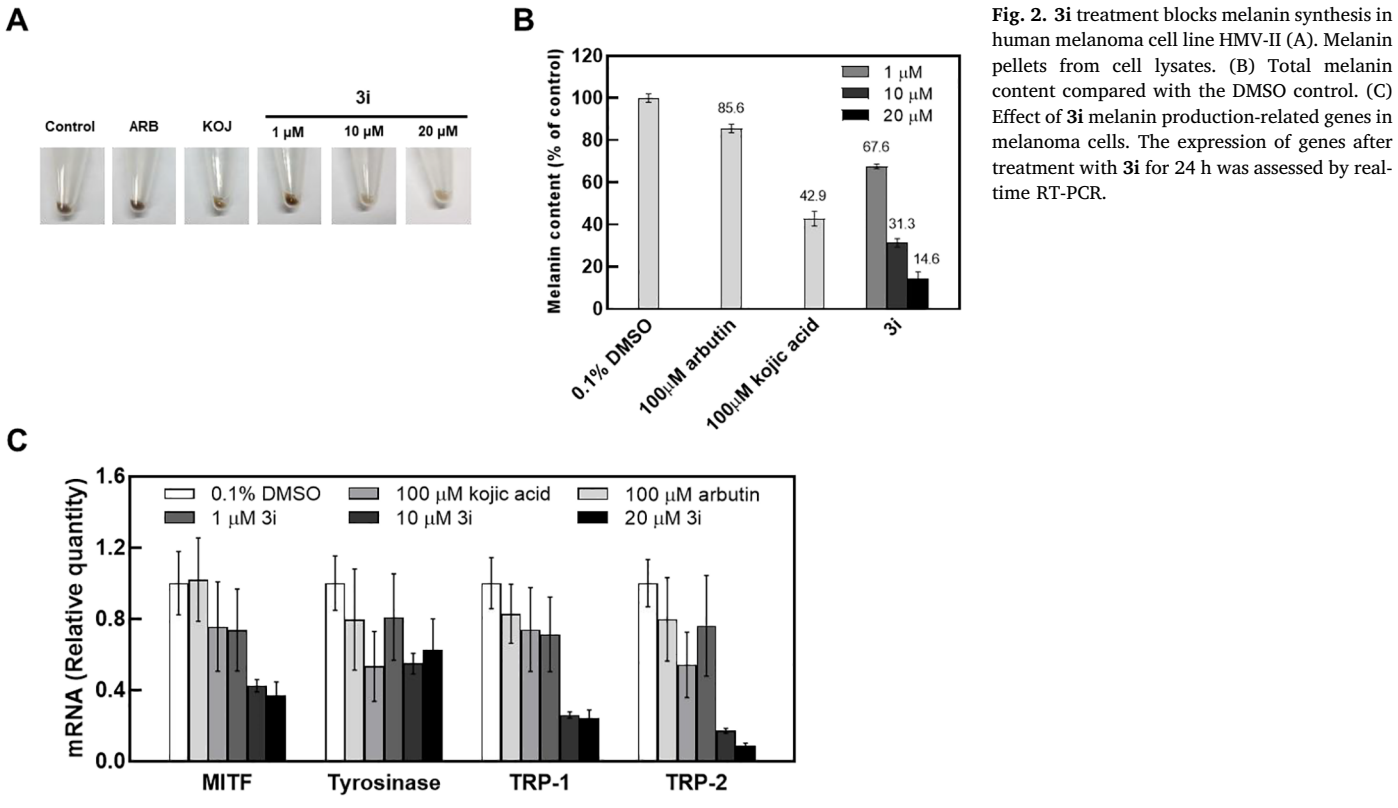


Fig. 2. 3i treatment blocks melanin synthesis in human melanoma cell line HMV-II (A). Melanin pellets from cell lysates. (B) Total melanin content compared with the DMSO control. (C) Effect of **3i** melanin production-related genes in melanoma cells. The expression of genes after treatment with **3i** for 24 h was assessed by real-time RT-PCR.

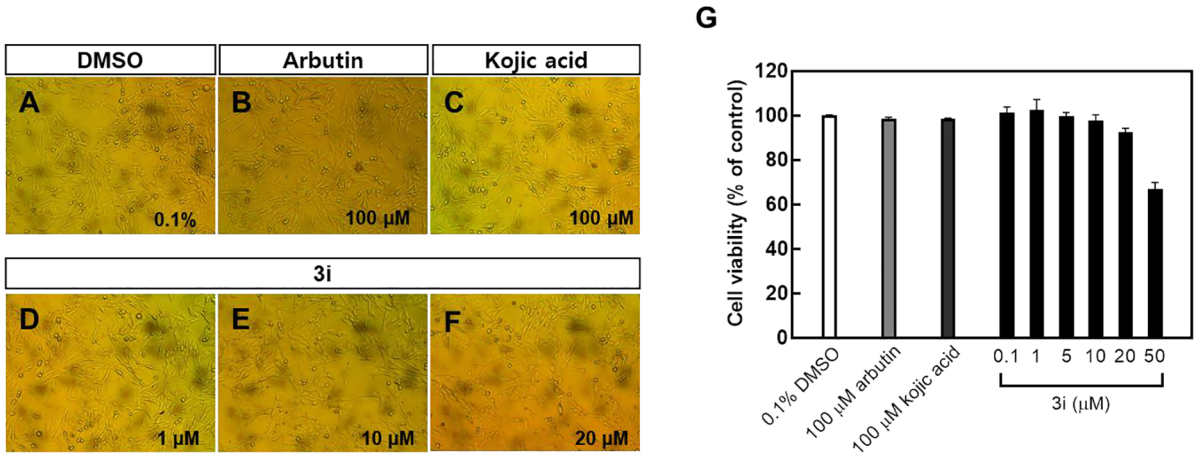


Fig. 3. Cytotoxic effect by **3i** was minimal in HMV-II cells up to 10 μM treatment. Cells were cultured 5 days with (A) DMSO, (B) Arbutin, (C) Kojic acid and (D-F) **3i**. HMV-II cells were seeded in 96-well plates at a density of 7,500 cells/well and cultured for 24 h. The cells were treated with each sample for (G) 5 days. CytoX reagent was used to measure cell vability.

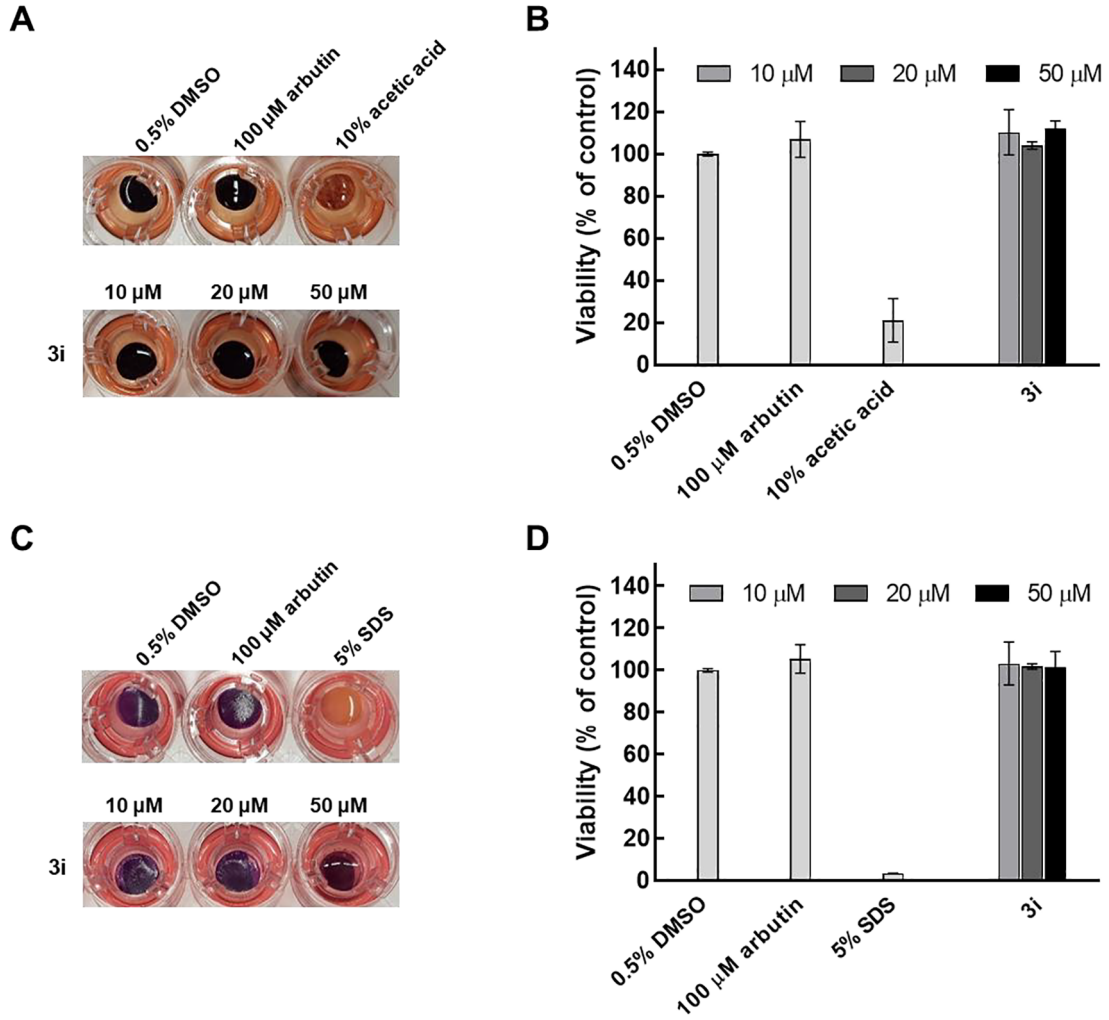


Fig. 4. Effects of **3i** on the viability of artificial ocular and skin tissue. (A) The artificial ocular tissues were stained with MTT solution (1 mg/mL) after each treatment for 30 min. (B) Formazan was extracted with isopropanol from the tissues and absorbance was measured at 570 nm using a microplate reader (M1000pro; Tecan). (C) The artificial skin tissues were stained with MTT solution (0.3 mg/mL) after each treatment for 3 h. (D) Formazan was extracted with 0.04 N isopropanol from the tissues and quantified with a microplate reader.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127201.

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