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Journal Name

ARTICLE

Design, Synthesis and Antimetastatic Evaluation of 1-benzothiazolylphenylbenzotriazoles for Photodynamic Therapy in Oral Cancer Cells

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We have designed and synthesized a new series of 1-benzothiazolylphenylbenzotriazoles **9a-p** and studied their antimetastatic mechanism involved in photosensitive effects induced by UVA in oral cancer cell Ca9-22. Our results revealed that compound plus UVA significantly suppressed the migration and invasion as detected by wound healing assay and Boyden chamber assay. Quantitative RT-PCR assay indicated that compound **9i** plus UVA induced antimetastatic effect through up-regulation of syndecan-1 and TIMP-3 and down-regulation of heparanase, MMP-2, and MMP-9 mRNA expressions. Western blot analysis showed that Ca9-22 treated by **9i** plus UVA resulted in decreased levels of p-EGFR and p-ERK, MMP-2 and MMP-9, increased level of TIMP-3. These results are the first to report the function of UVA-activated 1-benzothiazolylphenylbenzotriazoles in tumor metastasis and its underlying molecular mechanism, and thus suggesting compound **9i** plus PDT may serve as a potential ancillary modality for the treatment of oral cancer.

Introduction

Oral cancer is the sixth most common type of cancer in the world.[1] It has a tendency to metastasis to regional or distant sites after incomplete treatment. Photodynamic therapy (PDT) might be a potential therapeutic modality for oral cancer.[2,3] It is recognized as a treatment strategy which is both minimally invasive and toxic for cancer cells. Most modern PDT applications involve three key components: a photosensitizer, a light source and tissue oxygen. The wavelength of the light source needs to be appropriate for exciting the photosensitizer to produce oxygen free radicals, which are responsible for the PDT-induced cytotoxicity.[4] A previous study indicated that tumor tissues have higher uptake or retention of photosensitizer than the surrounding normal tissue.[5] However, there were also limitations of PDT, including the penetration depth for both photosensitizer and its activating light source. These limitations have prompted us to synthesize new photosensitizers.

We have previously reported 2-(4-aminophenyl)-benzothiazole derivatives as a photosensitizers for PDT on BCC cells[6] and later we found that the 2-(4-aminophenyl)benzothiazole compounds containing halogen groups, under UVA light exposure, induced A375 melanoma cell apoptosis.[7] Moreover, our research group have also found that bicyclic[1,2,3]triazoles protect UVA-induced senescence-like characteristics in fibroblast cells, which may provide potential prevention against photoaging.[8a] On the other hand, there is a significant growth towards the design and development of new therapeutic agents for the treatment of cancers.[8b] In this context, various benzotriazole derivatives have been found to possess excellent anticancer activity,[8c] for example, commercial available anticancer drug 4,5,6,7-tetrabromobenzotriazole (TBB)[8d] Furthermore, structurally diversified benzothiazoles and their metal complexes have shown the potentiality to overcome limitations of available clinical drugs.[8e] By considering the importance of benzotriazoles and our previously reported 2-(4-aminophenyl)benzothiazole.[6] Hereby, we describe the synthesis of a new class of 1-benzothiazolylphenylbenzotriazoles compounds which has an absorption in the UVA region. The synthesized compounds have been studied for the *in vitro* analyses on oral cancer cells (Ca9-22) for anti-migration effect under UVA irradiation. One can speculate that UVA will produce side effects such as carcinogenesis and photoaging, but in our system, these do not appear because the exposure time required for 1-benzothiazolylphenylbenzotriazoles activation was very short and did not lead to chronic exposure to UVA.

Matrix metalloproteinases (MMPs) are a family of secreted or transmembrane zinc endopeptidases that are capable of

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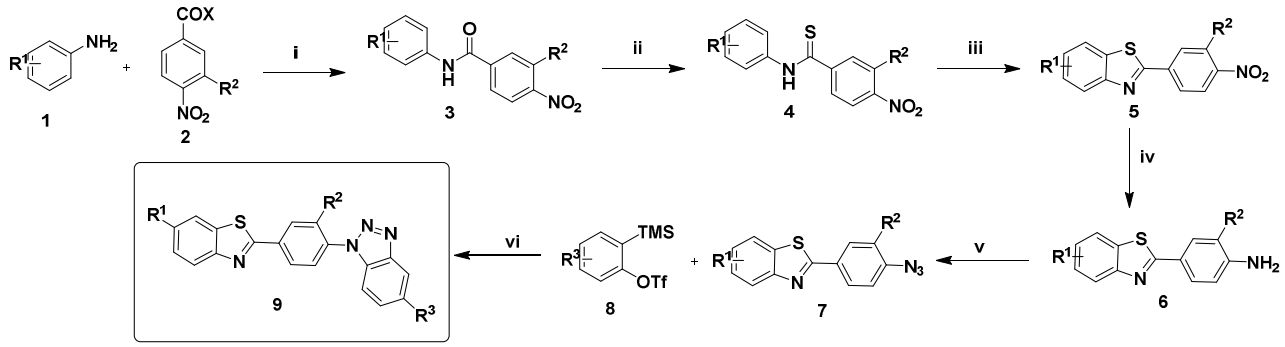
digesting extracellular matrix (ECM).[9] Among human MMPs, MMP-2 and MMP-9 are mostly associated with tumor migration and invasion for various cancers.[10] Additionally, clinical studies have also shown that MMP-2 and MMP-9 are involved in tumor metastasis and their elevated expression has been with poor prognosis.[11,12] Therefore, inhibition of MMP activity is important for arresting metastasis. Syndecan-1 has been shown to be a prognostic factor in various types of tumors, suggesting its correlation with malignancy and metastasis. Syndecan-1 is known to suppress the MMP-9 level in Ca9-22 cells[13] and can be degraded by heparanase.[14] Heparanase can enhance tumor cell growth and migration and induce the phosphorylation of epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK).[15] In this regard, we have initiated experiments aimed at characterizing the above signaling molecules in the process of antimetastatic after Ca9-22 cells were treated with 2-phenylbenzothiazoles-linked-benzotriazoles plus UVA.



9a, R^1 = H; R^2 = H; R^3 = H.
9b, R^1 = H; R^2 = H; R^3 = MeO.
9c, R^1 = Et; R^2 = H; R^3 = H.
9d, R^1 = Et; R^2 = H; R^3 = MeO.
9e, R^1 = Et; R^2 = CH_3; R^3 = H.
9f, R^1 = Et; R^2 = CH_3; R^3 = MeO.
9g, R^1 = CF_3; R^2 = H; R^3 = H.
9h, R^1 = CF_3; R^2 = H; R^3 = MeO.
9i, R^1 = MeO; R^2 = H; R^3 = H.
9j, R^1 = MeO; R^2 = H; R^3 = MeO.
9k, R^1 = H; R^2 = CH_3; R^3 = H.
9l, R^1 = H; R^2 = CH_3; R^3 = MeO.
9m, R^1 = MeO; R^2 = CH_3; R^3 = H.
9n, R^1 = MeO; R^2 = CH_3; R^3 = MeO.
9o, R^1 = CF3; R^2 = CH_3; R^3 = H.
9p, R^1 = CF3; R^2 = CH_3; R^3 = MeO.

Scheme 1. Total synthesis of 1-benzothiazolylphenylbenzotriazoles **9**. Reagents and conditions: (i) method a: X = Cl, pyridine, reflux; method b: X = OH, $SOCl_2$, benzene, reflux. (ii) Lawesson's reagent, chlorobenzene, 135 °C. (iii) $K_3Fe(CN)_6$ aq, NaOH, 90 °C. (iv) H_2/Pd/C 10%, CH_2Cl_2, 25 °C. (v) $NaNO_2$, NaN_3, 2N HCl (vi). CsF, CH_3CN, 55 °C, 12 h.

Results and Discussion

Syntheses

The synthetic strategy for the newly proposed series of 1-benzothiazolylphenylbenzotriazoles **9** was shown in Scheme 1. The synthetic protocol for the construction of compounds **6** was carried out according to our previously published literature protocol in good to excellent yields.[6] Compounds **7** was obtained by reaction of compounds **6** with $NaNO_2$ followed by azidation with NaN_3. The target compounds **9** was obtained by a slightly modified protocol of Larock et al[16a] with 2-(trimethylsilyl)phenyl trifluoromethanesulfonates **8** and azides **7** with CsF[16b] as base in CH_3CN under heating condition (55 °C). Compounds **9a**-**p** were isolated in good yields and characterized as described in experimental part.

Cytotoxicity of 2-phenylbenzothiazoles-linked-benzotriazoles plus UVA to Ca9-22 cells

The effect of UVA-activated 1-benzothiazolylphenylbenzotriazoles on Ca9-22 cell viability was evaluated by the MTT assay. Cells were cultured with the agents at 5 μM for 4 h before 1 J/cm^2 UVA irradiation. Twenty-four hours after irradiations, cell viability was measured for 16 compounds as shown in Table 1. The data revealed that compound **9e**, **9i** and **9k** shown to possess ≥50 % of inhibition as compared with other agents. Hence, we decided to study further biological studies of these 3 compounds compared with the analogues **9f**, **9j** and **9l**. The difference between these two sets is the presence of methoxy group (MeO-) in the aromatic ring. However, these methoxy derivatives shown no inhibition and it may be due to the strong electron-donating nature of methoxy group, which can potentially undergo further chemical transformation under UVA irradiation. From Table 1 and Figure 1, cell viability was significantly inhibited at 1J/cm^2 UVA irradiation. To evaluate the effects of PDT on the migration and invasion of cancer cells, a sublethal dose that killed about 20% of the cells was used (See Figure 1). Thus, the concentration and light dose we used throughout this study was 2 μM and 0.5 J/cm^2. Additionally, our data also showed that **9i** plus UVA exhibited a higher inhibitory activity more than either UVA irradiation alone or treatment with exogenous 9i alone.

1-benzothiazolylphenylbenzotriazoles plus UVA suppressed the migration and invasion of Ca9-22 cells

Migration[17a] and invasion of cancer cells are key steps of tumor metastasis. To investigate the inhibitory effect of 1-benzothiazolylphenylbenzotriazoles plus UVA on migration of Ca9-22 cells, the wound healing assay was performed. The

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Table 1. Effect of compounds plus UVA on Ca9-22 cell viability[a]

entry	Compound 9 +UVA	Survival (%)
1	UVA alone	100 ±0.2
2	**9a**	95.5±0.4
3	**9b**	104.4±0.2
4	**9c**	108.7±0.7
5	**9d**	113.6±0.7
6	**9e**	50.2±0.6
7	**9f**	73.1±0.2
8	**9g**	91.8±0.1
9	**9h**	104.9±0.2
10	**9i**	44.1±0.3
11	**9j**	81.1±0.8
12	**9k**	48.6±0.2
13	**9l**	79.6±0.7
14	**9m**	82.1±0.1
15	**9n**	63.3±0.3
16	**9o**	89.4±0.6
17	**9p**	112.9±0.2

[a]Cells were cultured with compounds 9a-9p at 5 μM for 4 h before 1 J/cm^2 UVA irradiation. Twenty-four hours after irradiation, cell survival was assessed using the MTT assay. The data are expressed as the mean ± SD.

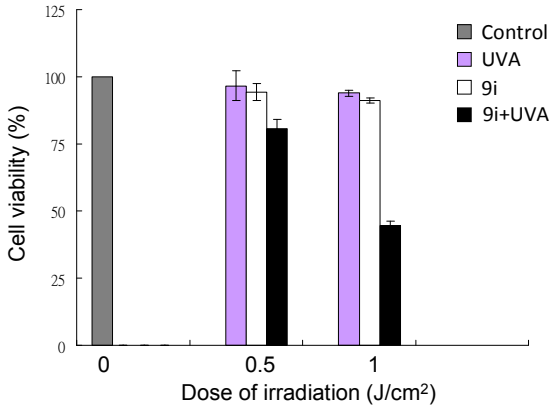
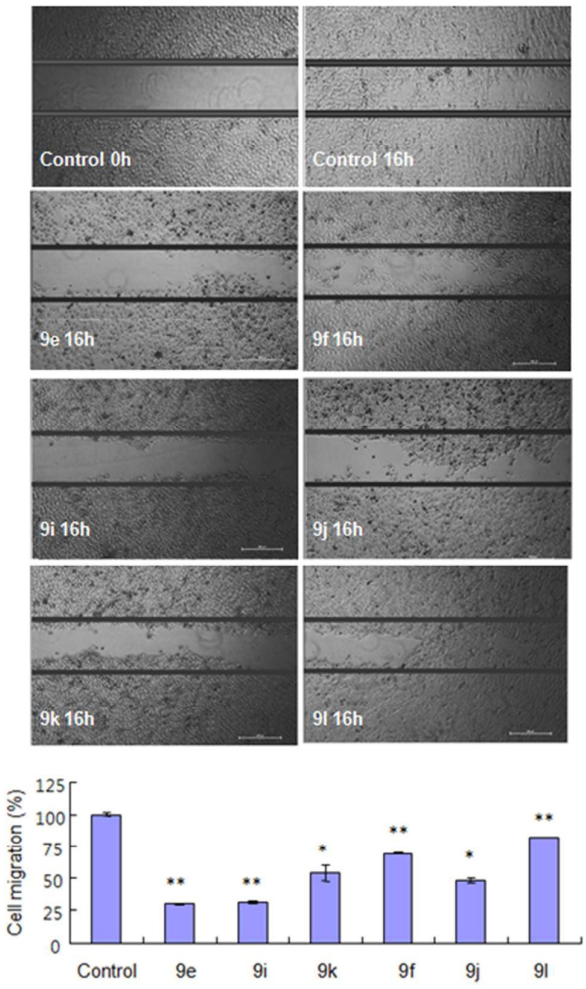


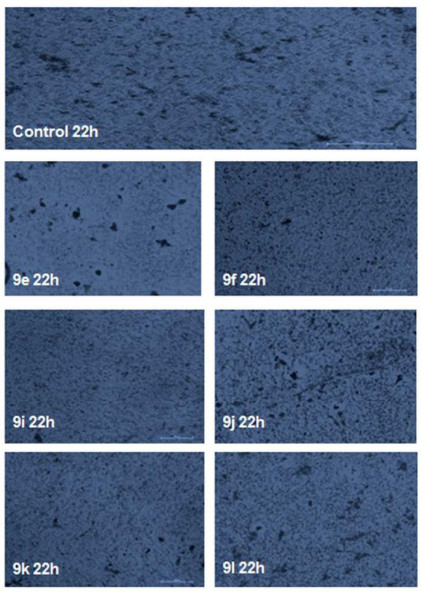
Figure 1. Effect of compound **9i** plus UVA cell viability. Ca9-22 cells were cultured with or without 2 μM **9i** before different dosage of UVA irradiation. Similar results were obtained in three independent experiments.

confluent monolayer was scraped by a sterile micropipette tip to create a scratch wound. Ca9-22 cells were treated with 2 μM agents for 2 h before 0.5 J/cm^2 UVA irradiation. Sixteen hours after irradiation, the cells migrated to the denuded zone and the level of the wound closure area was analyzed. As shown in Figure 2A, treatment of Ca9-22 cells with agents plus UVA inhibited 70.1% (**9e**), 68.7% (**9i**), 45.8% (**9k**), 31.1% (**9f**), 51.7% (**9j**) and 18.5% (**9l**) of cell migration, respectively. To further examine the effect of agents plus UVA on the invasive ability of Ca9-22 cells, a Boyden chamber coated with Matrigel was used. The results showed that the number of cells invading the lower chamber was 47.0% (**9e**), 38.9% (**9i**), 47.6% (**9k**), 70.0% (**9f**), 108.6% (**9j**) and 75.9% (**9l**) after 2 μM agents plus UVA, respectively, compared with that of control group (Figure. 2B).

A.



B.



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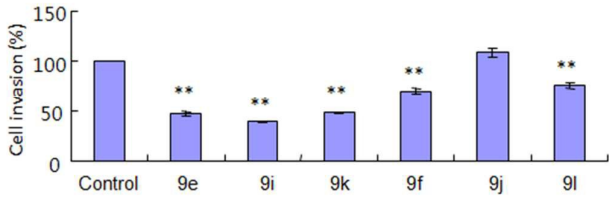

Figure 2. Effect of 1-benzothiazolylphenylbenzotriazoles plus UVA on the migration and invasion of Ca9-22 cells. (A) 5×10^5 cells were seeded on a 12-well plate, and cells were scraped to create a clean 1 mm wide wound area, Cells then were treated with the agents at 2 μM for 2h before 0.5 J/cm^2 UVA irradiation. Sixteen hours after irradiation, the wound areas were then analyzed and calculated using an online image analysis softwareWimasis. (B) In the Boyden chamber invasion assay, cells were treated with 2 μM agents for 2 h then 0.5 J/cm^2 UVA irradiation. Twenty-two hours after irradiation, the invasion ability of cells was quantified by counting the number of cells that invaded the undweside of the porous polycarbonate membrane under microscope. *$p < 0.05$, ** $p < 0.01$ as compared with the control group. Similar results were obtained in three independent experiments.

Metastasis-associated gene expression

Matrix metalloproteinases (MMPs) are highly expressed in invasive tumors and the levels of MMP-2 and MMP-9 were related to invasion of oral cancer.[10] Syndecan-1 is a trans-membrane heparan sulfate proteoglycan that involve in processes such as cell growth, differentiation and adhesion.[17b] Syndecan-1 is known to suppress the MMP-9 level in Ca9-22 cells[13] and can be degraded by heparanase.[14] Therefore, we used the qPCR measurements for syndecan-1, heparanase, MMP-2, MMP-9, and TIMP-3 (MMP inhibitor) expressions after **9i** plus UVA treatment. As shown in Figure 3, the mRNA expression of syndecan-1 and TIMP-3 had significant increase after **9i** plus UVA treatment, while the levels of heparanase, MMP-2, MMP-9 were reduced, as compared with the control group.

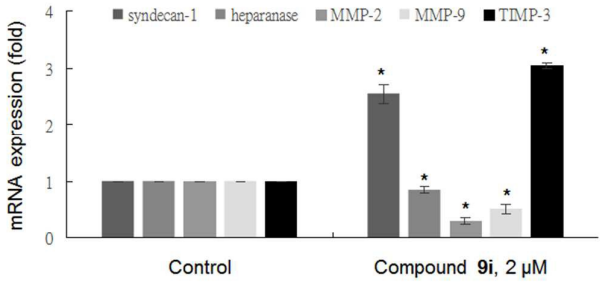

Figure 3. Expression of syndecan-1, heparanase, MMP-2, MMP-9, and TIMP-3 mRNA in different groups. The mRNA expression analyzed by quantitative real-time PCR and the expressional levels were normalized to the level of β-Actin mRNA. *p<0.05 vs control group. Similar results were obtained in three independent experiments.

Metastasis-associated protein expression

The epidermal growth factor receptor (EGFR) is expressed at high levels in most oral squamous cell carcinoma [18,19] and associated with lower rates of survival.[20] It has shown that ERK is over expressed in many malignant tumor tissues and cell lines, such as hepatocellular carcinoma, breast adenocarcinoma, and squamous cell carcinoma of head and neck, indicating that abnormal regulation of the ERK pathway is closely related to the tumorigenesis and development of malignant tumors.[21-23] To examine whether agent plus UVA-induced anticellular migration involves the regulation of the expression of EGFR, ERK and MMPs, Ca9-22 cells treated with 2 μM agents for 2 h then 0.5 J/cm^2 UVA irradiation were subjected to the Western blotting assay. Our results showed that 2-phenylbenzothiazoles-lined-benzotriazoles plus UVA inhibiting metastasis cell was associated with decreased levels of p-EGFR, p-ERK, MMP-2 and MMP-9, whereas the levels of TIMP3 were increased (Figure. 4).

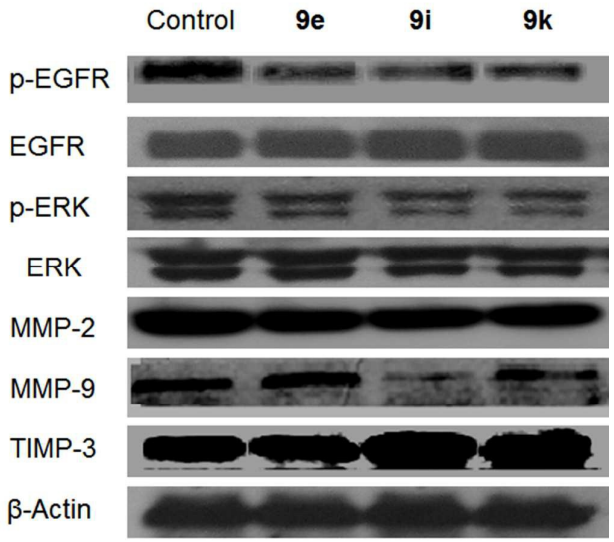

Figure 4. Western blot analysis showed the effect of compounds on the expression of metastasis regulatory proteins. After exposure to UVA with agents, cell lysates were collected and immunoblotted with specific antibodies as indicated. For the internal control, the same amounts of protein extract were also probed with antibody against β-Actin. Similar results were observed in three separate experiments.

Conclusions

We have discovered new series of benzothiazoles linked benzotriazoles by new synthetic strategy. The signaling mechanism of oral cancer cells Ca9-22 antimetastasis induced by 2-phenylbenzothiazoles-linked-benzotriazoles plus UVA treatment was disclosed. Our data revealed that compounds **9** plus UVA significantly inhibited the migration and invasion of Ca9-22. Data from quantitative RT-PCR assay indicated that compound **9i** plus UVA induced suppressive effect through up-regulation of syndecan-1 and TIMP-3 and down-regulation of heparanase, MMP-2, and MMP-9 mRNA expressions. Furthermore, compound **9** plus UVA decreased protein expression of p-EGFR, p-ERK, MMP-2 and MMP-9, increased level of TIMP-3. Taken together, our studies indicate that **9i** plus UVA inhibited the activity of both MMP-2 and MMP-9 via EGFR/ ERK signaling pathway in Ca9-22 cells. These findings reveal a new therapeutic potential for 1-benzothiazolylphenylbenzotriazoles -PDT on anti-metastatic therapy.

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Experimental Section

Chemistry

Materials and Methods Chemical reagents were used without further purification. Solvents free distilled prior to use. Reactions were monitored by thin layer chromatography, using Merck plates 60 F254. Flash chromatography was carried out on Merck Silica Gel 60 (40–63 lm) using the indicated solvents. Melting points were determined using Fargo MP-2D and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian UNITY plus-400 at 400 and 100 MHz, respectively, using $CDCl_3$ as a solvent. ^{1}H NMR chemical shifts are referenced to TMS or $CDCl_3$ (7.26 ppm). ^{13}C NMR was referenced to $CDCl_3$ (77.0 ppm). Mass spectra were recorded with Bruker APEX II spectrometer. Elemental analyses were performed on Elementar vario EL III analyzer, and the results were found to be ±0.4% of the theoretical values. Purity of tested compounds was >95%.

The experimental procedures to obtain compound **6** was followed according to our previously published literature protocol.[6]

General procedure for the synthesis of compound 9a-9p. To a stirred solution of 2- (4-aminophenyl) benzothiazole **6** in 2N HCl was added sodium nitrite (2.0 equiv) at 0 – 5 °C and stirred for 10 minutes followed by the addition of sodium azide (3.0 equiv) at the same temperature and allowed to stir until the completion of the starting material. The completion of the reaction was monitored by TLC. After completion, the reaction was diluted with water and extracted in ethyl acetate, dried over Na_2SO_4 and concentrated under vacuum to get the desired azide compounds **7**. To the crude compounds **7**, 2.0 equiv of 2-(Trimethylsilyl)phenyl trifluoromethanesulfonates **8** was added followed by the addition of 2.0 equiv of CsF and heated at 55 °C for 12h in acetonitrile as solvent. The completion of the reaction was monitored by TLC. After completion, the reaction was diluted with water and extracted with dichloromethane and dried over Na_2SO_4 and concentrated to afford the final compounds **9a**-**9p** in excellent yields.

1-(4-Benzothiazol-2-yl-phenyl)-1*H*-benzotriazole (9a). Yield 95%; White solid; mp = 218~220 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.35 (dt, *J* = 8.4 Hz, 2H), 8.19 (d, *J* = 8.4 Hz,1H), 8.12 (d, *J* = 8.0 Hz, 1H), 7.98 (dt, *J* = 8.4 Hz, 2H),7.95 (d, *J* = 8.0 Hz, 1H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.62 (t, *J* = 7.2 Hz, 1H), 7.54 (t, *J* = 7.2 Hz, 1H), 7.48 (t, *J* = 7.2 Hz, 1H), 7.44 (t, *J* = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.24, 154.13, 146.70, 138.86, 135.16, 133.64, 132.01, 128.99 (2C), 128.64, 126.61, 125.60, 124.66, 123.45, 122.82 (2C) , 121.72, 120.58, 110.34; HRMS (ESI+, m/s) for $C_{19}H_{13}N_4S$, calcd: 329.0861, found: 329.0859; anal. calcd for: $C_{19}H_{12}N_4S$; C, 69.49; H,3.67; N, 17.06 found: C, 69.49; H, 3.68; N, 17.08.

1-(4-Benzothiazol-2-yl-phenyl)-5-methoxy-1*H*-benzotriazole (9b). Yield 90%; White solid; mp = 186~188 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.34 (d, *J* = 8.8 Hz, 1H),8.33 (d, *J* = 8.8 Hz,1H), 8.12 (d, *J* = 7.6 Hz, 1H), 8.02 (d, *J* = 9.6 Hz, 1H), 7.94 (t, *J* = 8.8 Hz, 3H), 7.54 (t, *J* = 7.6 Hz, 1H), 7.43 (t, *J* = 7.6 Hz,1H), 7.26 (d, *J* = 6.4 Hz, 1H), 7.1 (d, *J* = 8.4 Hz, 2H), 3.93 (d, *J* = 6.4 Hz , 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.26 (d,*J*=166,1C), 160.91, 154.11, 141.91, 138.92, 135.15, 133.53, 133.30, 128.98(d,*J*=3.8,2C), 126.59, 125.59, 123.44, 122.92 (2C), 122.52, 121.72, 121.16, 116.62, 55.83 (d, *J* = 11.4, 1C) HRMS (ESI+, m/s) for $C_{20}H_{15}N_4OS$, calcd: 359.0967, found: 359.0964; anal. calcd for: $C_{20}H_{14}N_4OS$; C, 67.02; H, 3.94; N, 15.63 found: C, 65.86; H, 3.91; N, 15.34.

1-[4-(6-Ethyl-benzothiazol-2-yl)-phenyl]-1*H*-benzotriazole (9c). Yield 92%; White solid; mp = 190~192 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.33 (dt, *J* = 8.8 Hz, 2H),8.19 (d, *J* = 8.4 Hz,1H),8.02 (d, *J* = 8.0 Hz, 1H), 7.96 (dt, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 1H), 7.76 (s, 1H), 7.61 (t, *J* = 7.2 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.38 (dd, *J* = 8.0 Hz, 1H), 2.82 (q, *J* = 7.6 Hz, 2H), 1.34 (t, *J* = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 165.28, 152.44, 146.69, 142.29, 138.65, 135.40, 133.84, 132.04, 128.86 (2C), 128.61, 127.20, 124.65, 123.07, 122.83 (2C), 120.57, 120.26, 110.36, 28.99, 15.79; HRMS (ESI, m/s) for $C_{21}H_{17}N_4S$, calcd: 357.1174, found: 357.1173; anal. calcd for: $C_{21}H_{16}N_4S$; C,70.76; H,4.52; N, 15.72 found: C, 69.88; H,4.52; N, 15.43.

1-[4-(6-Ethyl-benzothiazol-2-yl)-phenyl]-5-methoxy-1*H*-benzotriazole (9d). Yield 85%; Yellow solid; mp = 200~202 °C; ^{1}H NMR ($CDCl_3$, 400 MHz); δ 8.32 (d, *J* = 8.8 Hz, 1H),8.31 (d, *J* = 8.8 Hz, 1H), 8.01 (d, *J* = 8.4 Hz, 1H), 7.93 (t, *J* = 9.2 Hz, 2H), 7.76 (s, 1H) , 7.69 (d, *J* = 9.2 Hz,1H), 7.48 (s,1H) 7.37 (d, *J* = 8.0 Hz, 1H), 7.26 (dd, *J* = 9.2 Hz, 1H), 7.09 (d, *J* = 8.8 Hz, 1H), 3.93 (d, *J* = 8.0 Hz, 3H), 2.82 (q, *J* = 7.6 Hz, 2H) , 1.33 (t, *J* = 7.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 165.27, 160.91, 157.55, 152.46, 147.85, 142.27, 138.70, 135.41, 133.76, 128.83 (2C), 127.17, 123.06, 122.54 (2C), 121.16, 120.25, 116.61, 111.01, 55.88, 29.98, 15.75 HRMS (ESI+, m/s) for $C_{22}H_{19}N_4OS$, calcd: 387.1280, found: 387.1281; anal. calcd for: $C_{22}H_{18}N_4OS$; C, 68.37; H, 4.69; N, 14.50 found: C, 67.41; H, 4.66; N, 14.23.

1-[4-(6-Ethyl-benzothiazol-2-yl)-2-methyl-phenyl]-1*H*-benzotriazole (9e). Yield 86%; White solid; mp = 150~152° C; ^{1}H NMR ($CDCl_3$, 400 MHz); δ 8.21 (s, 1H), 8.18 (d, *J* = 8.4 Hz,1H), 8.08 (dd, *J* = 8.4 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 7.54 (s, 1H), 7.53 (dd, *J* = 8.0 Hz, 2H), 7.45 (t, *J* = 7.2 Hz, 1H), 7.40 (d, *J* = 8.4 Hz,1H), 7.37 (dd, *J* = 8.4 Hz,1H), 2.81 (q, *J* = 7.6, 2H), 2.68 (s, 3H); 1.33 (t, *J* = 7.8, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 165.33, 152.36, 145.67, 142.29, 136.88, 135.98, 135.41, 135.07, 133.67, 130.37,128.25, 127.31, 127.15, 125.95, 124.27, 123.04, 120.22, 120.16, 109.97, 28.93, 17.99, 15.72; HRMS (ESI+, m/s) for $C_{22}H_{18}N_4SNa$, calcd: 393.1150, found: 393.1148 anal. calcd for: $C_{22}H_{18}N_4S$; C, 71.32; H, 4.90; N, 15.12 found: C, 71.41; H, 4.91; N, 15.08.

1-[4-(6-Ethyl-benzothiazol-2-yl)-2-methyl-phenyl]-5-methoxy-1*H*-Benzotriazole (9f). Yield 85%; White solid; mp = 160~162 °C; ^{1}H NMR ($CDCl_3$, 400 MHz); δ 8.20 (s, 1H), 8.08 (d, *J* = 8.0 Hz, 1H),8.02 (d, *J* = 8.4 Hz, 1H), 7.76 (s, 1H),7.52 (d, *J* = 8.0 Hz, 1H),7.48 (s, 1H), 7.38 (d, *J* = 8.4 Hz, 1H),7.27 (d, *J* = 8.4 Hz,1H), 7.20 (d, *J* = 8.8 Hz, 1H), 3.94 (s, 3H),2.82 (q, *J* = 7.6 Hz, 2H), 2.28 (s, 3H), 1.33 (t, *J* = 7.6 Hz,3H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 165.39, 157.46, 152.41, 146.75, 142.32, 136.99, 135.90, 135.45, 135.05, 130.40, 129.24, 127.24, 127.19, 125.97, 123.09, 120.97, 120.25, 110.63, 98.95, 55.79, 28.97, 18.01,15.75; HRMS (ESI+, m/s) for $C_{23}H_{21}N_4OS$, calcd:

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401.1437, found: 401.1431; anal. calcd for $C_{23}H_{20}N_4OS$: C, 68.98; H, 5.03; N, 13.99 found: C, 68.87; H, 4.99; N, 13.90.

1-[4-(6-Trifluoromethyl-benzothiazol-2-yl)-phenyl]-1*H*-benzotriazole (9g). Yield 84%; Yellow solid; mp = 272~274 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.38 (d, *J* = 8.4 Hz, 2H), 8.26 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 2H), 8.02 (d, *J* = 8.8 Hz, 2H), 7.85 (d, *J* = 8.4 Hz, 1H),7.78 (d, *J* = 8.8 Hz, 1H), 7.63 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 146.77, 139.48, 135.21, 132.95, 131.97, 129.30 (2C), 128.78, 124.78, 124.26 (q, *J* = 292.5, CF_3), 123.78, 123.63, 123.60, 122.86 (2C), 120.69, 119.47, 119.43, 114.21, 110.31; HRMS (ESI, m/s) for $C_{20}H_{12}N_4S$, calcd: 397.0730, found: 397.0729; anal. calcd for: $C_{20}H_{11}F_3N_4S$; C, 60.60; H, 2.80; N, 14.13 found: C, 59.00; H, 2.77; N, 13.73.

5-Methoxy-1-[4-(6-trifluoromethyl-benzothiazol-2-yl)-phenyl]-1*H*-Benzotriazole (9h). Yield 84%; White solid; mp = 228~230 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.37 (d, *J* = 8.8 Hz, 1H), 8.36 (d, *J* = 8.8 Hz, 1H), 8.25 (s, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 8.03 (dd, *J* = 9.6 Hz, 1H), 8.01 (s, 1H), 7.97 (d, *J* = 8.8 Hz, 1H), 7.75 (dd, *J* = 8.4 Hz, 1H), 7.27 (d, *J* = 9.2 Hz, 1H), 7.11 (dd, *J* = 8.4 Hz, 1H), 3.94 (d, *J* = 5.6 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.34, 160.99, 157.60, 156.02, 141.96, 139.51 (d, *J* = 5.3, 1C), 135.20, 132.83, 129.28(d, *J* = 3.8 ,2C), 125.44 (q, *J* = 270.6, CF_3), 123.76, 123.60 (d, *J* = 3.8, 1C), 122.94 (2C), 122.56, 121.26, 119.04 (d, *J* = 3.8, 1C), 116.64, 110.95, 55.85 (d, *J* = 11.3, 1C) HRMS (ESI+, m/s) for $C_{21}H_{14}F_3N_4OS$, calcd: 427.0840, found: 427.0843; anal. calcd for $C_{21}H_{13}F_3N_4OS$: C, 59.15; H, 3.07; N, 13.14 found: C, 58.87; H, 3.11; N, 13.04.

1-[4-(6-Methoxy-benzothiazol-2-yl)-phenyl]-1*H*-benzotriazole (9i). Yield 86%; Yellow solid; mp = 176~178 °C; ^{1}H NMR ($CDCl_3$, 400 MHz); δ 8.29 (dt, *J* = 9.2 Hz, 2H),8.18 (dt, *J* = 8.4 Hz, 1H),7.99 (dd, *J* = 9.2 Hz, 1H), 7.95 (dt, *J* = 8.8 Hz, 2H), 7.83 (dt, *J* = 8.4 Hz, 1H),7.61 (d, *J* = 7.6 Hz, 1H), 7.48 (t, *J* = 8.0 Hz, 1H), 7.39 (d, *J* = 2.4 Hz, 1H), 7.13 (dd, *J* = 9.2 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz); δ 163.67, 158.07, 148.68, 146.66, 138.45, 136.59, 133.81, 132.02, 128.63 (2C), 128..59, 124.63, 123.95, 122.83 (2C), 120.54, 116.03, 110.35, 104.13, 55.83; HRMS (ESI, m/s) for $C_{20}H_{15}N_4OS$, calcd: 59.0958, found: 359.0961; anal. calcd for: $C_{20}H_{14}N_4OS$; C, 67.02; H, 3.94; N, 15.63 found: C, 66.84; H, 3.94; N, 15.60.

5-Methoxy-1-[4-(6-methoxy-benzothiazol-2-yl)-phenyl]-1*H*-Benzotriazole (9j). Yield 81%; White solid; mp = 192~194 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.27 (t, *J* = 8.4 Hz, 2H), 7.97 (t, *J* = 9.2 Hz, 1H), 7.91 (t, *J* = 8.0 Hz, 2H), 7.7 (d, *J* = 8.8Hz, 1H), 7.46 (s, 1H) , 7.11 (m, 2H), 7.97 (d, *J* = 8.8 Hz,1H), 7.75 (dd, *J* = 8.4 Hz,1H), 7.27 (d, *J* = 9.2 Hz, 1H), 3.92 (s, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.24, 160.54, 157.67, 157.16, 148.28, 138.08, 136.22, 133.30, 132.92, 128.25, 123.50, 122.54, 122.13, 120.60, 116.36, 115.68, 110.72, 103.77, 98.83, 90.52, 55.46; HRMS (ESI+, m/s) for $C_{21}H_{17}N_4O_2S$, calcd: 389.1072, found: 389.1071.

1-(4-Benzothiazol-2-yl-2-methyl-phenyl)-1*H*-benzotriazole (9k). Yield 86%; White solid; mp = 156~158 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 (s, 1H), 8.19 (dt, *J* = 7.6 Hz, 1H),8.12 (t, *J* = 7.2 Hz, 2H), 7.96 (d, *J* = 7.2 Hz, 1H), 7.57-7.52 (m, 3H), 7.48-7.40 (m, 3H), 2.29 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.37, 154.09, 145.69, 137.14, 136.09, 135.22, 134.94, 133.68, 130.57, 128.31, 127.41, 126.61, 126.14, 125.64, 124.33, 123.48, 121.74, 120.24, 109.99, 18.05; HRMS (ESI+, m/s) for $C_{20}H_{14}N_4SNa$, calcd: 365.0837, found: 365.0839; anal. calcd for: $C_{20}H_{14}N_4S$; C,70.15; H, 4.12; N, 16.36 found: C, 70.03; H, 4.13; N, 16.21.

1-(4-Benzothiazol-2-yl-2-methyl-phenyl)-5-methoxy-1*H*-benzotriazole (9l). Yield 83%; White solid; mp = 122~124 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.23 (s, 1H), 8.11 (t, *J* = 8.4 Hz, 2H),7.96 (dt, *J* = 6.8 Hz,1H), 7.54 (d, *J* = 6.4 Hz, 2H),7.49 (d, *J* = 2.4 Hz, 1H), 7.45 (t, *J* = 7.0 Hz, 1H), 7.27 (d, *J* = 3.2 Hz, 1H), 7.20 (dd, *J* = 8.8 Hz, 1H), 3.94 (s, 3H), 2.92 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 166.36, 160.77, 157.45, 154.08, 146.75, 140.95, 137.18, 135.96, 135.21, 130.55, 127.51, 126.01, 125.63, 124.71, 123.47, 116.69, 110.61, 98.93, 90.05, 55.81; HRMS (ESI+, m/s) for $C_{21}H_{17}N_4OS$, calcd: 373.1119, found: 373.1118.

1-[4-(6-Methoxy-benzothiazol-2-yl)-2-methyl-phenyl]-1*H*-Benzotriazole (9m). Yield 81%; white solid; mp = 176~178 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.18 (dt, *J* = 8.0 Hz, 2H), 8.05 (dd, *J* = 8.4 Hz, 1H), 8.03 (d, *J* = 8.8 Hz, 1H), 7.54 (t, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.0 Hz, 1H), 7.46 (t, *J* = 8.0 Hz,1H), 7.40 (dt, *J* = 9.2 Hz, 2H), 7.14 (dd, *J* = 9.2 Hz,1H), 3.91 (s, 3H), 2.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.75, 158.07, 148.62, 145.65, 136.69, 136.64, 135.99, 135.07, 133.67, 130.15, 128.26, 127.35, 125.76, 124.29, 123.96, 120.19, 116.06, 109.99, 104.08, 55.81, 18.02; HRMS (ESI+, m/s) for $C_{21}H_{16}N_4OSNa$, calcd: 395.0942, found: 395.0945; anal. calcd for: $C_{21}H_{16}N_4OS$; C, 67.72; H, 4.33; N, 15.04 found: C, 67.53; H, 4.33; N, 15.03.

5-Methoxy-1-[4-(6-methoxy-benzothiazol-2-yl)-2-methyl-phenyl]-1*H*-benzotriazole (9n). Yield 79%; White solid; mp = 162~164 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.17 (s, 1H), 8.04 (t, *J* = 6.4 Hz, 1H), 8.01 (d, *J* = 9.2 Hz, 1H), 7.51 (d, *J* = 8.0 Hz,1H), 7.48 (s, 1H), 7.40 (s, 1H), 7.27 (d, *J* = 6.8 Hz, 1H),7.20 (d, *J* = 8.8 Hz,1H), 7.14 (d, *J* = 8.8 Hz, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.94 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 163.79, 160.75, 158.10, 157.45, 148.66, 146.73, 136.66, 135.09, 130.17, 127.25, 125.85, 123.98, 120.98, 116.68, 116.06, 110.03, 104.13, 98.92, 90.06, 55.83, 55.79, 18.00; HRMS (ESI+, m/s) for $C_{22}H_{19}N_4O_2S$, calcd: 403.1230, found: 403.1223; anal. calcd for $C_{22}H_{18}N_4O2S$: C, 65.65; H, 4.51; N, 13.92 found: C, 65.56; H, 4.56; N, 13.86.

1-[2-Methyl-4-(6-trifluoromethyl-benzothiazol-2-yl)-phenyl]-1*H*-Benzotriazole (9o). Yield 84%; White solid; mp = 188~190 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.26 (s, 2H), 8.21 (d, *J* = 7.2 Hz, 1H), 8.20 (dt, *J* = 8.0 Hz, 1H), 8.15 (dd, *J* = 8.4 Hz, 1H), 7.78 (dd, *J* = 8.4 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 7.47 (t, *J* = 7.2 Hz, 1H),7.42 (dt, *J* = 8.4,1H), 2.31 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.46, 155.97, 145.72, 137.75, 136.30, 135.26, 134.26, 133.61, 130.86, 128.42, 127.88, 127.52, 126.65 (q, *J* = 244.8, CF_3); 126.37, 124.42, 123.61, 123.56, 120.31, 119.47, 109.93, 18.14; HRMS (ESI+, m/s) for $C_{21}H_{14}F_3N_4S$, calcd: 411.0891, found: 411.0893; anal. calcd for: $C_{21}H_{13}F_3N_4S$; C, 61.46; H, 3.19; N, 13.65 found: C, 61.43; H, 3.19; N, 13.66.

5-Methoxy-1-[2-methyl-4-(6-trifluoromethyl-benzothiazol-2-yl)-phenyl]-1*H*-benzotriazole (9p). Yield 83%; white solid; mp = 158~160 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.30 (s, 1H), 8.26 (s, 1H), 8.21 (d, *J* = 8.4 Hz, 1H), 8.16 (dd, *J* = 8.0 Hz, 1H), 7.78 (dd, *J* = 8.8 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.49-7.47 (m, 1H),7.31 (d,

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J = 9.2 Hz, 1H), 7.22 (dd, *J* = 8.8 Hz, 1H), 3.95 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.99, 157.01, 155.46, 146.19, 137.24, 135.55, 134.81, 133.64, 130.34, 128.60, 127.09, 126.88, 125.87, 123.62 (q, *J* = 270.6, CF_3), 123.27, 123.01 (d, *J* = 3.8, 1C), 120.61, 119.04, 110.15, 98.39, 55.31, 17.58; HRMS (ESI+, m/s) for $C_{22}H_{16}F_3N_4OS$, calcd: 441.0998, found: 441.0991.

Biology

Cell culture. Human gingival carcinoma Ca9-22 cells were cultured in DMEM-F12 medium (Gibco, Grand Island, NY). Cells were supplemented with 10% fetal bovine serum (FBS), 100 U/mL penicillin, 100 μg/mL streptomycin, 0.03% glutamine, and 1mMsodiumpyruvate. Cells were kept at 37◦C in a humidified atmosphere containing 5% CO_2.

UVA irradiation. For UVA irradiation, a specific UVA lamp emitting a peak wavelength of 365 nm (UVP, Upland, CA, USA) was used. The cultured cells were pre-treated with different agents at 2 μM for 2 h before UVA irradiation. The cultured cells were rinsed with phosphate-buffered saline (PBS) and then irradiated with UVA in PBS to avoid the formation of medium-derived toxic photoproducts induced by UV exposure. The doses of irradiation were measured by a UVX digital radiometer (UVP, Upland, CA, USA) and the incident irradiance at the surface of the cells was found to be 4.762 mW/cm^2 at the target distance of 17 cm. The calculation formula of designated time for UVA treatment is energy (J/cm^2) = power (W/cm^2) × exposure time (s). Immediately after photo treatment, PBS was removed and media were added to the cells. All the following experiments were performed three times in triplicate.

Cell viability. Cell viability was assessed by the MTT assay, a mitochondrial function assay based on the ability of viable cells to reduce the redox indicator MTT to insoluble formazan crystals by mitochondrial dehydrogenase. Briefly, cells were seeded in a 96-well plate at the cell density of 10000 cells/well. After an overnight incubation, the cells were treated with compounds at 2 μM for 2 h followed by 0.5 J/cm^2 UVA irradiation and incubated for 24 h. The medium was then discarded and replaced with 10 μL of MTT dye. Plates were incubated at 37 °C for 2 h. The resulting formazan crystals were solubilized in 100 μL DMSO, and the optical density was read at 540 nm with a microplate reader (MRX-II, Dynex technology, Chantilly, VA).

Wound healing assay. A total of 3 × 10^5 Ca9-22 cells were seeded onto 12-well plates and then grown to complete confluence. A 200 μL plastic pipette tip was used to scratch the culture monolayer and create a clean 1 mm wide wound area. Cells were treated with 2 μM agents for 2h followed by 0.5 J/cm^2 UVA irradiation. Sixteen hours after irradiation, wound gaps were photographed at each time interval. The wound areas were then analyzed and calculated using the online software Wimasis (Wimasis GmbH, Munich, Germany).

Cell invasion assay. The ability of Ca9-22 cells for passing through Matrigel-coated filters was measured by the Boyden chamber invasion assay. Cells were treated with 2 μM agents for 2h followed by 0.5 J/cm^2 UVA irradiation. Twenty two hours after irradiation, cell were detached by trypsin and resuspended in serum-free medium. Medium containing 10% FBS was added to the lower chamber as chemo attractant and then the cells were seeded on the upper chamber at a density of 1 × 10^5 cells/well in 300 μL of serum-free medium. The chamber was incubated for 24 h at 37 °C. At the end of incubation, the cells in the upper surface of the membrane were carefully removed with a cotton swab and cells invaded across the Matrigel to the lower surface of the membrane were fixed with methanol and stained with haematoxylin and eosin. The invasive cells on the lower surface of the membrane filter were counted with a microscope.

Quantitative RT-PCR. Total RNA was extracted from Ca9-22 cells with 2 μM 9i for 2h followed by 0.5 J/cm^2 UVA irradiation. Eight hours after irradiation and the Trizol reagent (Invitrogen) was used. Two micrograms of total RNA were used for reverse transcription using RevertAidTM H Minus First Strand cDNA Synthesis Kit (Fermentas, MA, USA) following the manufacturer's instructions. For real-time qPCR, the ABI PRISM 7900 Sequence Detection System (ABI) was used. Nine microlitres of master-mix (2X Maxima® SYBR Green/ROXqPCR Master Mix, 0.3 μM forward primer, 0.3 μM reverse primer) and 1 microlitre of 100 nanograms cDNA were added to the 96-well plates and amplified using a suitable program. At the completion of cycling, melting curve analysis was performed to establish the specificity of the amplicon production. Data were analyzed according to the comparative Ct method and were normalized by β-Actin expression.

The primers used for amplification were as follows.
syndecan-1: forward, 5' – AGGACGAAGGCAGCTACTCCT - 3';
reverse, 5' – TTTGGTGGGCTTCTGGTAGG - 3'
heparanase: forward, 5' – AGAACAGCACCTACTCAAGAAGC - 3';
reverse, 5' – ATTCCCATTCGGGCTGACAGG - 3'
MMP-9: forward, 5' – GTGCTGGGCTGCTGCTTTGCTG - 3';
reverse, 5' - GTC GCCCTCAAAGGTTTGGAAT - 3'
MMP-2: forward, 5' – TCACATACAGGATCATTGGCTAC - 3';
reverse, 5' – GCCAGGAGTCCGTCCTTA - 3'
TIMP-3: forward, 5' – AAGCGATGTCAGAGGGCG - 3';
reverse, 5' – AACTGG ATGGGCAGCAGG - 3'
β-Actin: forward, 5'-GGAAATCGTGCGTGAC-3';
reverse, 5'-ATGCCCAGGAAGGAA-3'

Protein extraction and western blot analysis. Total cell extracts from cultured cells were obtained by lysing the cells in ice-cold RIPA buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA, 1 mM EGTA, 1% Triton X-100, and 0.25% sodium deoxycholate) containing 2 mM PMSF, 2 μg/mL aprotinin, 2 μg/ml leupeptin, 2 mM Na_3VO_4 and 2 mM NaF. After centrifugation at 14,000 rpm for 10 min, protein in the supernatants was quantified by Bradford method (Bio-Rad). Twenty micrograms of protein per lane was applied in 10% SDS-poly-acrylamide gel. After electrophoresis, protein was transferred from the gel to the polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA). The membranes were blocked at room temperature for 1 h in PBS + 0.1% Tween 20 (PBS-T) containing 5% skim milk. After briefly rinsing with PBS-T, the membrane was incubated with primary antibody at room temperature for 2 h or at 4 °C overnight.

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Rabbit polyclonal antibodies against EGFR, ERK and p-ERK, MMP-2, 9 and TIMP-3 were purchased from Santa Cruz Biotechnology (Santa Cruz , CA), Cell Signaling Technology (Beverly, MA), and Abgent (San Diego, CA), respectively. Goat polyclonal antibody against p-EGFR was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal antibody against β-Actin was purchased from Millipore (Billerica, MA). The membrane was incubated with the corresponding horseradish peroxidase-labeled secondary antibody (Santa Cruz Biotechnology) at room temperature for 1 h. Membranes were washed with PBS-T four times for 15 min, and the protein blots were visualized with Western Lightning Chemiluminescence Reagent Plus (Perkin-Elmer Life Sciences, Boston, MA). The relative amounts of specific proteins were quantified by densitometry scanning of X-ray films and analyzed by AlphaView Image software (Alpha Innotech Corporation, CA).

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References

1 R. Mishra, *Oral Oncol*. 2012, **48**, 578.
2 T. H. Yang, C. T. Chen, C. P. Wang and P. J. Lou, *Oral Oncol*. 2007, **43**, 358.
3 R. Bhuvaneswari, Q. F. Ng, P. S. Thong and K. C. Soo, *Oncotarget*. 2015, **6**, 13487.
4 D. E. Dolmans, D. Fukumura and R. K. Jain, *Cancer*. 2003, **3**, 380.
5 Q. Peng, K. Berg, J. Moan, M. Kongshaug and J. M. Nesland, *Photochem Photobiol*. 1997, **65**, 235.
6 W.-P. Hu, Y. K. Chen, C. C. Liao, H. S. Yu, Y. M. Tsai, S. M. Huang, F. Y. Tsai, H. C. Shen, L. S. Chang, J. J. Wang, *Bioorg. Med. Chem*. **2010**, *18*, 6197.
7 Y. -K. Chen, G. C. Senadi, C. -H. Lee, Y. -M. Tsai, Y. -R. Chen, W. -P. Hu, Y. -W. Chou, K. -K. Kuo and J. -J. Wang, *Chem. Res. Toxicol*. 2014, **27**, 1187.
8 a) H. -Y. Hsieh, W. -C. Lee, G. C. Senadi, W. -P. Hu, J. -J. Liang, T. -R. Tsai, Y. -W. Chou, K. -K. Kuo, C. -Y. Chen and J. -J. Wang, *J. Med. Chem*. 2013, **56**, 5422. b) Y. Ren, L. Zhang, C. –H. Zhou and R. –X. Geng, *Med. Chem*. 2014, 4, 640. c) S. Zhang, Y. Luo, L. –Q. He, Z. –J. Liu and A. –Q. Jiang, *Bioorg. Med. Chem*. 2013, **21**, 3723. d) J. S. Duncan, L. Gyenis, J. lenehan, M. Bretnar, L. M. Graves, T. A. Haystead, and T. W. Litchfield, Mol. Cell. Proteomics, 2008, 6, 1077. e) M. Entezar, M. safari, M. Hekmati, and A. Azin, *Med. Chem. Res.* 2014, **23**, 487.
9 P. L. Lee, H. V. Weelden and P. L. B. Bruijnzeel *Photochem. Photobiol*. 2008, **84**, 1528.
10 K. Kessenbrock, V. Plaks and Z. Werb, *Cell*. 2010, **141**, 52.
11 S. Chakraborti, M. Mandal, S. Das and A. Mandal, *Mol Cell Biochem*. 2003, **253**, 269.
12 E. I. Deryugina and J. P. Quigley, *Cancer Metastasis Rev*. 2006, **25**, 9.
13 T. Muramatsu, M. Saitoh, Y. Ro, T. Uekusa, E. Iwamura, K. Ohta, Y. Kohno, Y. Abiko and M. Shimono, *Oncol Rep*. 2008, **20**, 1353.
14 J. Reiland, R. D. Sanderson, M. Waguespack, S. A. Barker, R. Long, D. D. Carson and D. Marchetti, *J Biol Chem*. 2004, **279**, 8047.
15 V. Cohen-Kaplan, I. Doweck, I. Naroditsky, I. Vlodavsky, N. Ilan, Cancer Res. 2008, **68**, 10077.
16 a) F. Shi, J. P. Waldo, Y. Chen and R. Larock, *Org. Lett*. 2008, **10**, 2409. b) The reaction was carried out with various other fluoride bases such as TBAF, TBAT and also solvent and found CsF as best condition.
17 a) C. –C. Liang, A. Y. Park and J. L. Guan, Nature Protocols, 2007, **4**, 329. b) M. Bernfield, R. Kokenyesi, M. Kato, M. T. Hinkes, J. Spring, R. L. Gallo and E. J. Lose, *Annu Rev Cell Biol*. 1992, **8**, 365.
18 K. K. Ang, B. A. Berkey, X. Tu, H. Z. Zhang, R. Katz, E. H. Hammond, K. K. Fu and L. Milas, *Cancer Res*. 2002, **62**, 7350.
19 S. Takaoka, M. Iwase, M. Uchida, S. Yoshiba, G. Kondo, H. Watanabe, M. Ohashi, M. Nagumo and S. Shintani. *Int. J. Oncol*. 2007, **30**, 1469.
20 J. R. Grandis, M. F. Melhem, E. L. Barnes and D. J. Tweardy, *Cancer*. 1996, **78**, 1284.
21 Y. Ito, Y. Sasaki, M. Horimoto, S. Wada, Y. Tanaka, A. Kasahara, T. Ueki, T. Hirano, H. Yamamoto, J. Fujimoto, E. Okamoto, N. Hayashi and M. Hori, *Hepatology*. 1998, **27**, 951.
22 A. Adeyinka, Y. Nui, T. Cherlet, L. Snell and P. H. Watson, *Clin Cancer Res*. 2002, **8**, 1747.
23 J. Albanell, J. Codony-Servat, F. Rojo, J. M. Del Campo, S. Sauleda, J. Anido, G. Raspall, J. Giralt, J. Rosello, R. I. Nicholson, J. Mendelsohn and J. Baselga, *Cancer Res*. 2001, **61**, 6500.

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