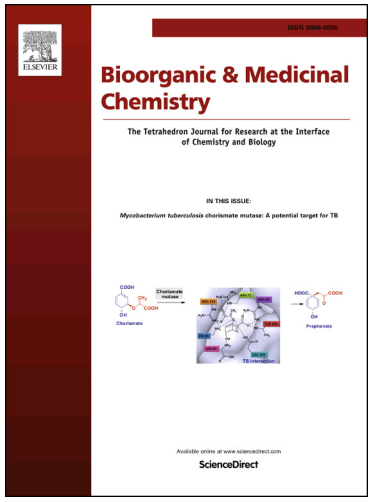
Accepted Manuscript



Design, synthesis, and biological evaluation of a highly water-soluble psoralen-based photosensitizer

Yoshiyuki Uruma, Takuya Nonomura, Priscilla Yoong Mei Yen, Marie Edatani, Ryotaro Yamamoto, Kunishige Onuma, Futoshi Okada

PII: S0968-0896(16)30832-X
DOI: http://dx.doi.org/10.1016/j.bmc.2017.02.050
Reference: BMC 13580

To appear in: *Bioorganic & Medicinal Chemistry*

Received Date: 28 September 2016
Revised Date: 24 February 2017
Accepted Date: 24 February 2017

Please cite this article as: Uruma, Y., Nonomura, T., Yen, P.Y.M., Edatani, M., Yamamoto, R., Onuma, K., Okada, F., Design, synthesis, and biological evaluation of a highly water-soluble psoralen-based photosensitizer, *Bioorganic & Medicinal Chemistry* (2017), doi: http://dx.doi.org/10.1016/j.bmc.2017.02.050

<Manuscript for Journal of Bioorganic Medicinal Chemistry>

<Title>

Design, synthesis, and biological evaluation of a highly water-soluble psoralen-based photosensitizer

<Author and Affiliation>

Yoshiyuki Uruma,*[1] Takuya Nonomura,[1] Priscilla Yoong Mei Yen,[1] Marie Edatani,[1] Ryotaro Yamamoto,[1] Kunishige Onuma,[2] and Futoshi Okada[2]

[1]Department of Materials Science, National Institute of Technology, Yonago College Yonago City, Tottori 683-8502
[2]Department of Biomedical Sciences, Tottori University, Yonago City, Tottori 683-8503

<Abstract>

In recent years, photodynamic therapy (PDT) has been approved for treating various medical conditions, including cancer. PDT is a treatment that employs a particular drugs, called photosensitizers which work along with specific light source. The growth of this medical industry is expanding as it is another promising alternative to treat cancer which lessen the burden of treatments in patients. This includes the benefits of minimally invasive procedures and delivering high accuracy in targeting mutations. In recent two decades, cancer researchers have produced remarkable studies on developing photosensitizers that enhance understanding of biology and genetics of cancer. It is unfortunate that not all PDT can work as well as other profound treatment because PDT has various limitations like PDT leads photosensitivity reaction that arises when the photosensitizer remains in the body for a long period of time. In this paper, our studies

centers on synthesizing a highly soluble photosensitizing agent with improved effectiveness on detecting cancer cells.

Introduction

In recent years, the mortality rate due to cancer has increased at an alarming rate, prompting the global scientific community to seek new treatments to control the disease. One of the more promising new treatments is photodynamic therapy (PDT).[1,2] PDT employs a type of drug called a photosensitizer, which is activated by light of a specific wavelength. The photosensitizer is injected into the bloodstream and absorbed by cells in the body. Then, the cancer cells are exposed to monochromatic light, causing the photosensitizer to produce singlet oxygen, which induces apoptosis in the cancer cells.[3] The light sources used for PDT can be coherent-wavelength lasers, which enable the treatment of organs and tissues in the body, or light-emitting diodes, that may be used for treating exterior tumors such as skin cancer.

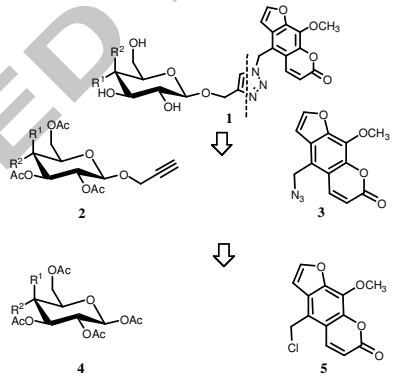
Unfortunately, most photosensitizers approved for PDT medication have drawbacks that limit their application in treating cancer, such as poor absorption, which leads to inefficient singlet-oxygen generation, and poor excretion from healthy cells. For instances, photofrin and psoralen are the approved and commonly used photodynamic therapy drugs as photosensitizers. Photofrin, also known as porfimer sodium is a

mixture of oligomers structured by eight units of porphyrin via ether and ester linkages. Nonetheless, photofrin exhibits absorption at low wavelength, poor excretion from healthy cells and low excitation yields in photocytotoxicity, it was approved to be practiced for the treatment in esophageal cancer. On the other hand, psoralen suppress apoptosis under ultraviolet (UVA) exposure, psoralen plus UVA (PUVA) therapy has indicated to be a considerable photosensitizer in treating skin cancer. Yet, similar in sound to what was intended, psoralen has poor solubility and selectivity for cancer cells, due to these adverse reactions, it is only used to treat early stage of skin cancer.[4] To overcome these drawbacks, we have developed glycoconjugate psoralen as photosensitizer in PDT therapy.[4] Since increasing glycolytic capacity in cancer cells activates Warburg effect, it is recognized that glycoconjugated psoralen has a better molecular design as photosensitizer because it may help develop the cancer cells selectivity.[5] Yano et al., among others also suggests that highly water-soluble photosensitizers exhibited improved rate of absorption and excretion therefore reduces the probability of drug side effects.[6,7,8] This paper presents the synthesis strategies of glycoconjugated psoralen, quantitation in terms of solubility via Q-NMR method and detailed analytic report on how increase in solubility effects singlet-oxygen production and photocytotoxicity.

Results and Discussion

Chemical Synthesis

The retrosynthetic analysis, shown in Scheme 1 envisaged that the target molecule **1** could be accessed via a triazole ring formation between **2** and **3** under Huisgen reaction conditions, in which a dipolarophile reacts with a 1,3-dipolar compound to afford a 5-membered cycle.

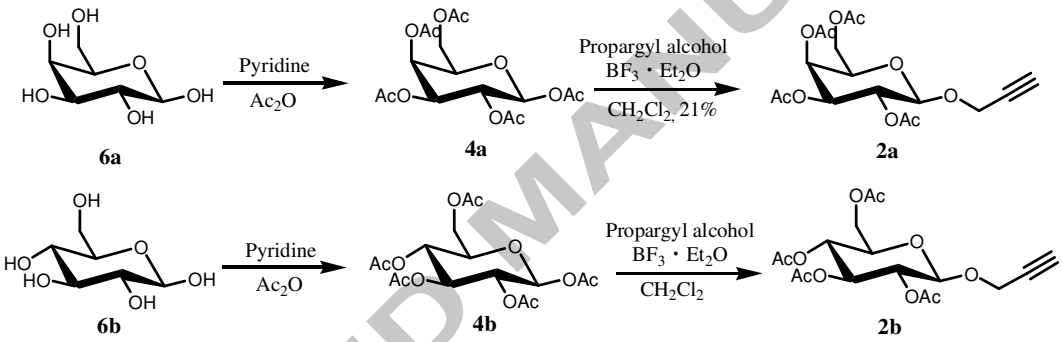


Scheme 1. Retrosynthetic analysis for glycoconjugated psoralen

To perform this reaction here, we employed the acetylene and azide functional groups of **2** and **3**, respectively. These key intermediates were obtained via the protected carbohydrate **4** and the chlorinated molecule **5**, respectively.[9]

We synthesized the acetylene derivatives **2a** and **2b** based on a previously reported method,[10] obtaining the galactose acetylene **2a** in 21% yield. Preparation of the glucose acetylene **2b** was accomplished with the crude product as **2b** was difficult to purify with silica gel column chromatography.

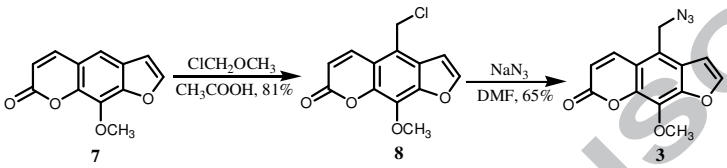
<Insert Scheme 2>



Scheme 2**.** Synthesis of acetylene derivatives

As direct introduction of the azide group into psoralen is an unconcreted pathway, compound **7** was first converted to a chlorinated compound **8** by using chloromethyl methyl ether[11].

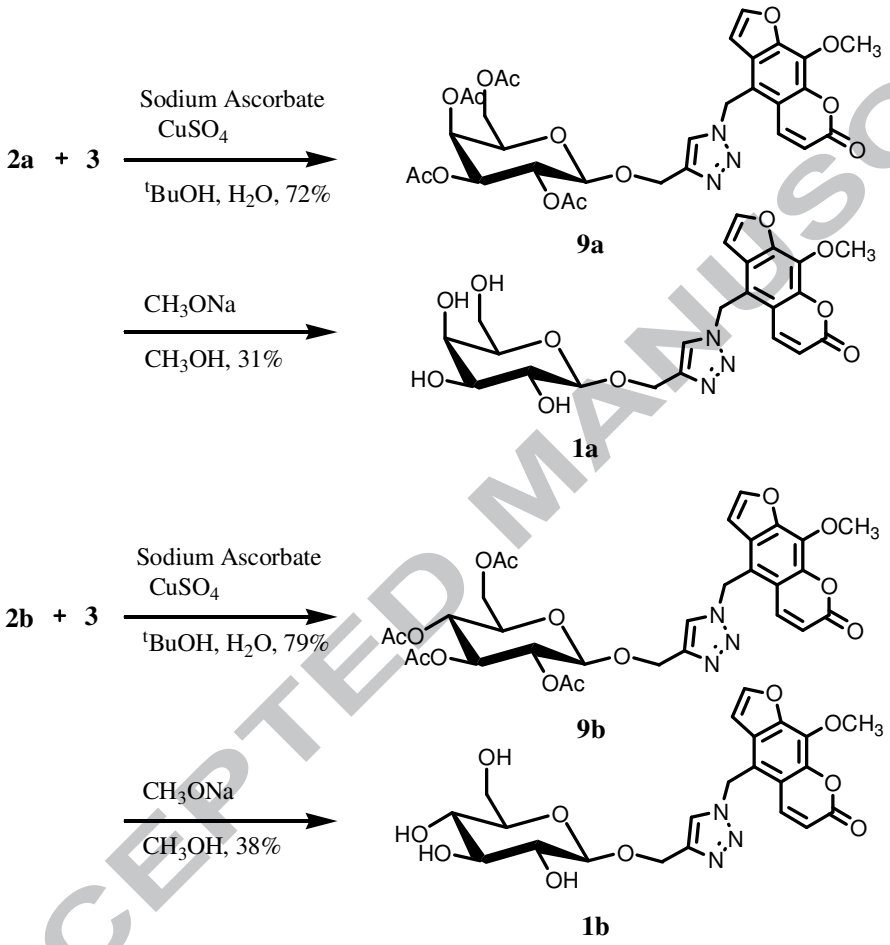
<Insert Scheme 3>



Scheme 3. Synthesis of psoralen derivatives

The chlorinated substituent was utilized in the synthesis of the psoralen azide derivative through nucleophilic substitution where chlorinated substituent **8** was reacted with sodium azide to afford **3** as presented in scheme 3**.**

<Insert Scheme 4>



Scheme 4. Synthesis of glycoconjugated psoralen derivatives

Then, to prepare glycoconjugate psoralen derivative, heterocoupling between compound

3 and acetylene derivatives azide **2a** and **2b** by Huisgen reaction[12] is performed to obtain acetyl protected glyconjugate psoralen derivatives **9a** and **9b** shown in scheme 4. Finally, deprotection of the obtained acetyl protected coupling compound proceeded smoothly with sodium methoxide in methanol, affording **1a** and **1b** in 31% and 38% yields, respectively.

Evaluation of Synthetic Compounds

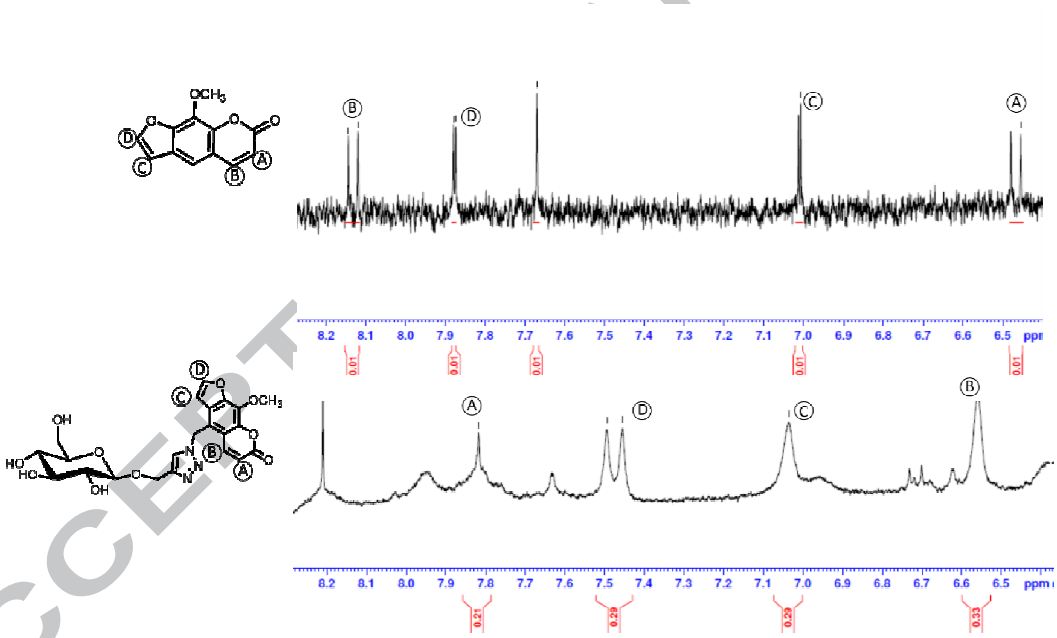
Assessment of singlet-oxygen production

The efficiency of converting ground state oxygen to singlet oxygen is an ultimate determinant used to evaluate the new PDT agents because the singlet oxygen has a unique chemistry that induces irreversible destruction of cancer cells within irradiated domain. In this examination, the samples, psoralen and glycoconjugated psoralen were dissolved in DMSO respectively and 1,3-diphenylisobenzofuran (DPBF), singlet oxygen acceptor which has an absorption maximum λ_{max} around 416 nm, was employed to monitor the production of singlet oxygen. The sample mixture was exposed under a constant light source of 50 W halogen lamp and the disappearance of DPBF was monitored spectroscopically.[13] The experimental results indicate that psoralen exhibits quantum yield of 17% and glycoconjugated psoralen shows improvement in singlet oxygen production with a quantum yield of 24%. From the obtained data, it is clearly implied that a glycoconjugated photosensitizer performs productively in PDT.

Water solubility test

The characterization of solubility of psoralen **7** and glycoconjugate psoralen **1b** were determined by using quantitative nuclear magnetic resonance method (Q-NMR), which is a relatively new spectroscopic method in this field[14] to assess the solubility of the drugs. Both samples were prepared using 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS-d_6) as an internal standard and dissolved in deuterated solvent D_2O.

<Insert Figure1>



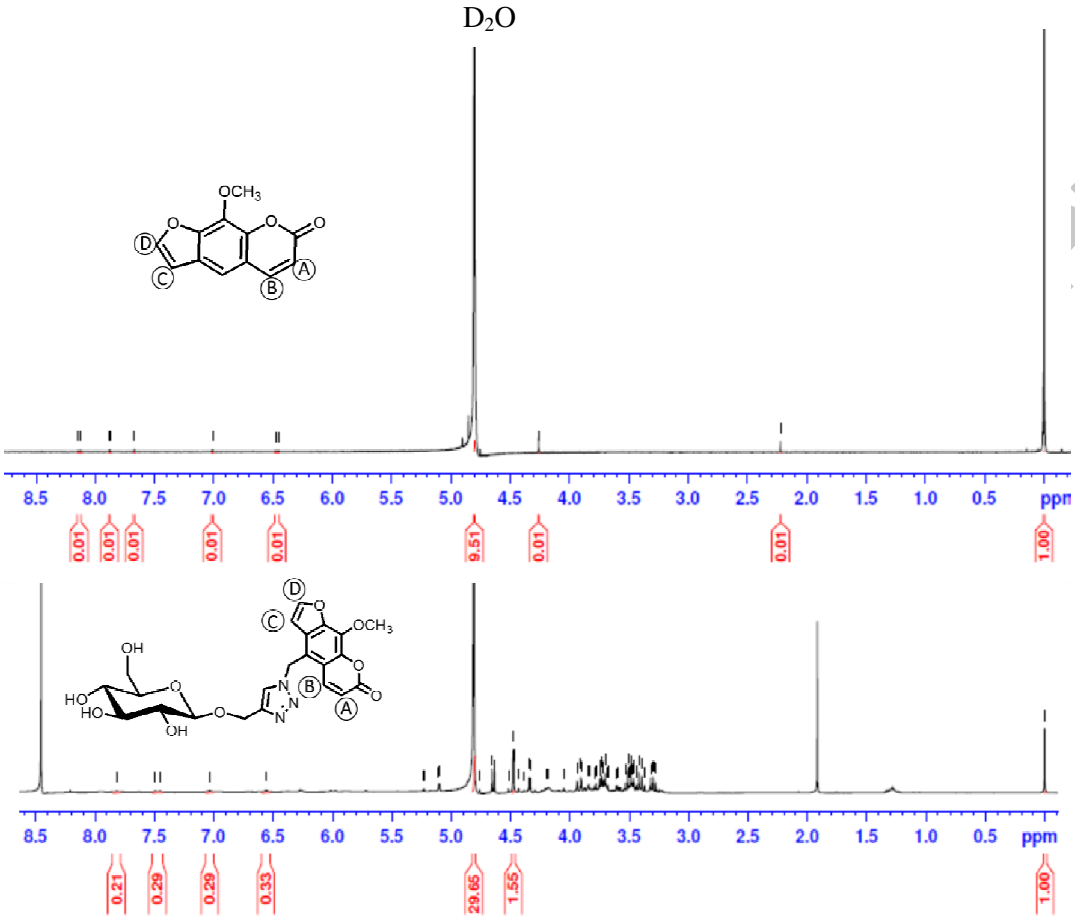


Figure 1 Q-NMR spectra of psoralen **7** and glucose-conjugated psoralen **1b**; (down) full spectrum (δ 8.50~0.00 ppm) and (up) magnification of aromatic region (δ 8.30~6.40 ppm). The aromatic peaks were indicated as A~D and were assigned based on the 2D NMR spectroscopy (HMBC and HMQC). Samples were prepared in D_2O and quantitation was evaluated using internal reference, DSS-d_6.

The Q-NMR analysis of psoralen and glucose-conjugated psoralen were shown in Figure 1. The singlet peak at δ 0.00 with integral value of 1.00 encodes the internal

reference, DSS-d_6 and was assigned as an analytic peak in calculating the solubility of dissolved compound. Several areas of the peaks of hydrogen atoms originated from the samples, without any overlapping were selected and labeled as A~D. The peaks A~D are signals aroused from aromatic ring of both samples, for psoralen are A: δ 6.47 (d, 1H, J = 9.6 Hz); B: δ 8.13 (d, 1H, J = 9.6 Hz); C: δ 7.01 (d, 1H, J = 2.4 Hz); D: δ 7.88 (d, 1H, J = 2.4 Hz) and for glucose-conjugated psoralen are A: δ 7.82 (br, 1H); B: δ 6.56 (br, 1H); C: δ 7.04 (br, 1H); D: δ 7.47 (d, 1H, J = 16 Hz). The irregular chemical shift of the aromatic protons of 1b was detected; peak A was shifted to downfield and peak D was shifted to upfield. The reason of the unexpected displacement was ambiguous. The average integral values of 4 peaks for psoralen and glucose-conjugated psoralen are 0.01 and 0.28 respectively. From the relationship of the known amount of DSS-d_6 and its integral area, the number of moles of dissolved psoralen and glucose-conjugated psoralen in deuterated solvent are 1.4×10^{-8} and 1.7×10^{-7} mol and therefore, the measurement indicates that glucose-conjugated psoralen is 12 times more soluble than psoralen .

Photocytotoxicity assay

The photocytotoxicity of psoralen **7** and its glycol-derivates, **1a** and **1b** were evaluated against BALB/3T3 cells after incubation for 24h at 37℃ in controlled environmental condition of humidified atmosphere containing 5% CO_2. Figure 2 shows the photoactivity of photosensitizers against percentage cell survival evaluated at light dose of 3.3 J/cm^2 upon incubation with photosensitizers for 48 h.

<Insert Figure2>

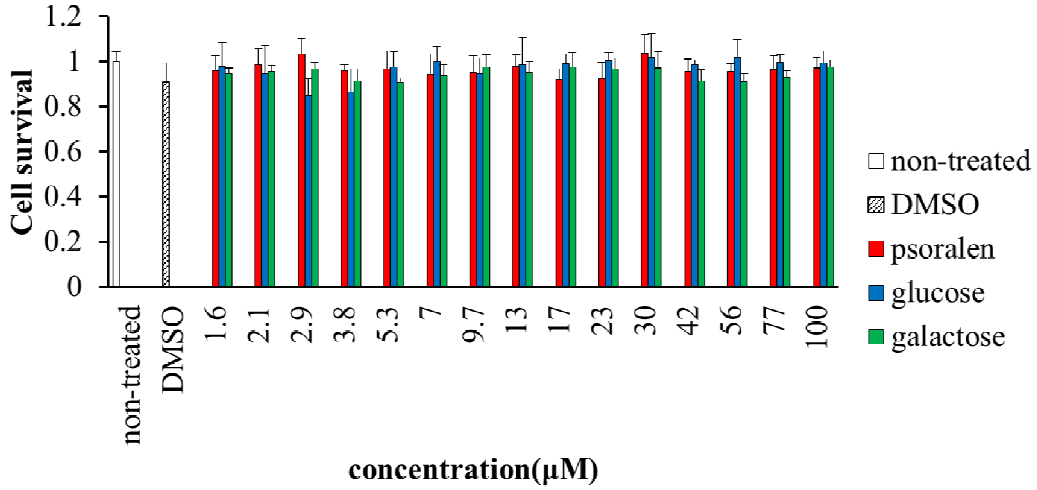


Figure 2 Percentage of cell survival ± S.D. towards different concentration of photosensitizers. Photocytotoxicity was determined against cancer cells, BALB/3T3 under light fluences rate of 3.3 J/cm^2 from the source of 365 nm UV light. The

incubation time with photosensitizers and irradiation time were kept constant, 48 h and 500 s respectively. Mean ± S.D of percentage of cell survival was analysed vs non treated (The bar denotes standard deviation ($P<0.05$) of mean, n=3). 'Non treated' indicates no addition of photosensitizer agents and 'DMSO' refers to solvent used.

Above result implied that at high concentration of photosensitizers (42 μM >), galactose conjugated psoralen exhibited a degree of enhancement in PDT activity compared to psoralen, but psoralen showed slightly higher apoptosis than glucose conjugated psoralen in spite of its high water solubility. At concentration between 7 and 23 μM, there was no major difference observed between two examined glycoconjugated photosensitizers, yet psoralen produced slightly better PDT effects. As reported previously by Hirohara et. al., [15] in general, increased molecular size and high concentration of photosensitizers suppress cellular uptake due to aggregation and subsequently lead to poor PDT effects. Hence, this could be the possible explanation for the unexpected results obtained at concentration 7~23 μM. However, at low concentration range from 2.1 to 5.3 μM, the data interprets that glycoconjugated psoralens showed improvement in photocytotoxicity. Particularly at concentration of 2.9

μM, glucose conjugated psoralen exhibited a significant contribution to PDT effect, inducing 15% cell death. It can be safely concluded that glycopyranosyl groups not only influence the amount of cellular uptake but also PDT effect[16], which support the hypothesis that high solubility photosensitizers promote photocytotoxicity.

Conclusion

The main conclusion of our experiments is that increased solubility of photosensitizers, evaluated based on the Q-NMR quantitation, promote singlet oxygen production and photocytotoxicity in low presence of drug. Further work on the photocytotoxicity assessment of analogues against several of cancer cells is underway to provide concrete guidelines and fundamentals for implementing in clinical practices.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a BRUKER 400 at 400 MHz and 100 MHz, respectively. The chemical shifts are reported as δ values using tetramethylsilane (0 ppm) and $CHCl_3$ (77.0 ppm) as internal standards for proton and carbon spectra, respectively. Infrared (IR) spectra were recorded on a JASCO FT/IR 420 spectrometer as liquid films on NaCl plates. Low- and high-resolution mass spectra (LRMS and HRMS, respectively) were obtained on JMS-AX 500 and JMS-700 T spectrometers at the Analytical Center of Osaka City University. Silica gel (silica gel 60, 230–400 mesh) was used for flash chromatography. Melting point was measured by MP-21 (Yamato Kagaku). Precoated silica gel plates (Merck 5715, 60F254) were used for thin-layer chromatography. All air-sensitive reactions were conducted in flame-dried glassware under Ar. Tetrahydrofuran, methylene chloride, dimethyl formamide (DMF), methanol, ethanol and ether were purchased from Wako (Japan).

Chemical syntheses

See Figures 1–2 for compound labels.

4-(azidomethyl)-9-methoxy-7H-furo[3,2-g]chromen-7-one (**3**). [17]

To a solution of **8** (387.4 mg, 1.47 mmol) in DMF (55 mL) was added NaN_3 (572.3 mg, 8.80 mmol) at r.t., and the reaction was stirred for 24 h. The reaction was then quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O and brine and then dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (CH_2Cl_2) to afford **3** (363 mg, 91%) as a white solid.

Rf = 0.4 (EtOAc:*n*-Hexane, 2:1); m.p. 154.0–156.0 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 4.31 (3H, s), 4.71 (2H, s), 6.45 (1H, d, *J* = 9.9 Hz), 6.94 (1H, d, *J* = 2.4 Hz), 7.73 (1H, d, *J* = 2.4 Hz), 8.02 (1H, d, *J* = 9.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 47.8, 61.3, 105.0, 115.4, 117.0, 126.9, 133.1, 139.9, 143.6, 146.6, 146.7, 147.1, 159.7; IR (NaCl) ν_{max} (cm^{-1}): 1714, 1421, 1362, 1221, 1092; HRMS (FAB, pos) *m/z* 272.0643 (calcd for $C_{13}H_{10}N_3O_4$, 272.0671 $[M+H]^+$)

Compound **9a**.

To a solution of **3** (151 mg, 0.6 mmol) in H_2O (2.8 mL) and *tert*-BuOH (2.8 mL) was

added acetylene derivative **2a** (258 mg, 0.7 mmol), l-(+)-ascorbic acid sodium salt (11 mg, 0.1 mmol), and copper (II) sulfate (18 mg, 0.1 mmol) at r.t., and the reaction was stirred for 24 h. The reaction was then quenched with H_2O and extracted with EtOAc. The combined organic layers were washed with H_2O and brine and then dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was chromatographed on silica gel (EtOAc) to afford compound **9a** (81 mg, 72%) as a white solid.

Rf = 0.42 (EtOAc); m.p. 95–96.0 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.85 (3H, s), 1.95 (3H, s), 2.02 (3H, s), 2.11 (3H, s), 3.89 (1H, td, *J* = 6.5 Hz, 1.0 Hz), 4.05 (1H, m), 4.12 (1H, m), 4.32 (3H, s), 4.57 (1H, d, *J* = 7.9 Hz), 4.72 (1H, d, *J* = 12.5 Hz), 4.86 (1H, d, *J* = 12.6 Hz), 4.97 (1H, dd, *J* = 10.4, 3.4 Hz), 5.13 (1H, dd, *J* = 10.5, 7.8 Hz), 5.36 (1H, dd, *J* = 4.4, 1.6 Hz), 5.91 (2H, s), 6.46 (1H, d, *J* = 9.9 Hz), 6.95 (1H, *J* = 2.3 Hz), 7.33 (1H, s), 7.78 (1H, d, *J* = 2.3 Hz), 8.16 (1H, d, *J* = 9.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.5, 20.6(3C), 21.0, 47.3, 60.4, 63.1, 66.9, 68.7, 70.7, 70.8, 100.6, 104.6, 115.1, 115.3, 116.3, 122.12, 127.1, 133.7, 139.2, 143.6, 145.2, 146.7, 147.7, 159.3, 169.4, 170.0, 170.1, 170.4; IR (NaCl) ν_{max} (cm^{-1}): 1751, 1714, 1590, 1422, 1363, 1222, 1162, 1132, 1091, 1046; HRMS (FAB, pos) *m/z* 658.1856 (calcd for $C_{30}H_{32}N_3O_{14}$, 658.1884 $[M+H]^+$)

Compound **9b**.

The procedure was the same as that used for the preparation of **9a** but proceeded from **2b** instead of **2a**. **9b** was prepared in 79% yield from corresponding compound (**2a** and **3**)

Rf = 0.42 (EtOAc); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.87 (3H, s), 1.96 (3H, s), 2.03 (3H, s), 2.11 (3H, s), 3.67 (1H, ddd, *J* = 9.7 Hz, 4.6 Hz, 2.4 Hz), 4.48 (1H, d, *J* = 7.9 Hz), 4.72 (1H, d, *J* = 12.5 Hz), 4.86 (1H, d, *J* = 12.6 Hz), 4.99 (1H, dd, *J* = 9.5 Hz, 8.0 Hz), 5.09 (1H, t, *J* = 12.4 Hz), 5.14 (1H, t, *J* = 9.3 Hz), 5.91 (2H, s), 6.46 (1H, d, *J* = 9.9 Hz), 6.95 (1H, d, *J* = 2.3 Hz), 7.78 (1H, d, *J* = 2.3 Hz), 8.16 (1H, d, *J* = 9.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 20.6(3C), 20.7, 47.2, 61.3, 61.6, 67.7, 68.2, 70.8, 71.9, 72.5, 100.5, 104.6, 114.8, 115.4, 116.3, 123.0, 127.1, 132.8, 139.1, 143.6, 146.7, 146.8, 159.3, 169.3(3C), 170.2, 170.4; IR (NaCl) ν_{max} (cm^{-1}): 1741, 1727, 1585, 1427, 1354, 1208, 1162, 1146, 1091; HRMS (FAB, pos) *m/z* 658.1886 (calcd for $C_{30}H_{32}N_3O_{14}$, 658.1884 $[M+H]^+$)

Compound **1a**.

To a solution of **9a** (73 mg, 0.1 mmol) in CH_3OH (1.1 mL) was added $NaOCH_3$ (12 mg, 0.2 mmol) at r.t., and the reaction was stirred for 30 min. The reaction was then evaporated *in vacuo*, and the residue was chromatographed on silica gel (EtOAc) to afford compound **1a** (17 mg, 31%) as a white solid.

Rf = 0.12 (EtOAc); m.p. 127.0–129.0 °C; ^{1}H NMR ($CDCl_3$ + CD_3OD, 400 MHz) δ 3.38 (1H, d, *J* = 3.3 Hz), 3.42 (1H, d, *J* = 3.2 Hz), 3.48 (1H, dd, *J* = 9.0, 7.3 Hz), 3.79 (1H, dd, *J* = 3.1, 0.8 Hz), 4.27 (3H, s), 4.71 (1H, d, *J* = 12.5 Hz), 4.88 (1H, d, *J* = 12.6 Hz), 6.06 (2H, s), 6.47 (1H, d, *J* = 9.9 Hz), 7.17 (1H, d, *J* = 2.4 Hz), 7.87 (1H, s), 7.91 (1H, d, *J* = 2.2 Hz), 8.46 (1H, d, *J* = 10.0 Hz); ^{13}C NMR ($CDCl_3$ + CD_3OD, 100 MHz) δ 47.8, 61.8, 62.3, 62.9, 70.0, 72.2, 74.7, 76.6, 104.0, 106.1, 115.9, 116.5, 117.9, 124.8, 128.7, 134.5, 142.0, 144.5, 146.2, 148.1, 149.0, 161.7; IR (NaCl) ν_{max} (cm^{-1}): 2045, 1449, 1422, 1115, 1031; HRMS (FAB, pos) *m/z* 490.1469 (calcd for $C_{22}H_{24}N_3O_{10}$, 490.1462 $[M+H]^+$)

Compound **1b**.

The procedure was the same as that used for the preparation of **1a** but proceeded from **9b**. **1b** was prepared in 38% yield from **9b**.

Rf = 0.12 (EtOAc); ^{1}H NMR (CD_3OD + $CDCl_3$, 400 MHz) δ 3.17 (1H, dd, *J* = 9.8, 8.0 Hz), 3.23 (1H, d, *J* = 1.8 Hz), 3.26 (1H, t, *J* = 9.5 Hz), 3.31 (1H, t, *J* = 8.5 Hz), 3.62 (1H, dd, *J* = 11.9, 5.4 Hz), 3.82 (1H, dd, *J* = 11.8, 1.9 Hz), 4.27 (3H, s), 4.31 (1H, d, *J* = 7.8 Hz), 4.71 (1H, d, *J* = 12.5 Hz), 4.88 (1H, d, *J* = 12.5 Hz), 6.06 (2H, s), 6.47 (1H, d, *J* = 10.0 Hz), 7.17 (1H, d, *J* = 2.1 Hz), 7.9 (1H, d, *J* = 2.2 Hz), 8.65 (1H, d, *J* = 10.0 Hz); ^{13}C NMR ($CDCl_3$ + CD_3OD, 100 MHz) δ 48.2, 61.4, 62.3, 62.4, 70.8, 72.2, 74.7, 76.6, 103.5, 106.7, 115.5, 116.3, 118.2, 124.8, 128.2, 133.5, 142.5, 144.5, 146.8, 148.7, 148.4, 162.42; IR (NaCl) ν_{max} (cm^{-1}): 2057, 1433, 1428, 1100; HRMS (FAB, pos) *m/z* 490.1472 (calcd for $C_{22}H_{24}N_3O_{10}$, 490.1462 $[M+H]^+$)

Biological Evaluation

Cell culture

BALB/3T3 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

Photocytotoxicity

The photocytotoxicities of the galactose- and glucose-conjugated photosensitizers against BALB/3T3 cells were investigated as follows: The cells (2.5×10^3 cells) in 100 μL DMEM containing 10% FBS were plated in a 96-well plate and incubated for 24 h (37 °C, 5% CO_2), and the required amount of photosensitizer in 100 μL of DMEM containing 10% FBS and 0.2–13.8 mM DMSO was added to each well. The photosensitizer concentration was varied from 1 to 500 μM, with the final DMSO content being 1% in all cases. The plate was then incubated for 48 h under normal oxygen condition. The cells were washed twice with brine, and then 100 μL of DMEM containing 10% FBS was added. The cells were exposed to 365 nm UV light for 500 s. The irradiation time was adjusted to obtain the desired light dose of 3.3 J cm^{-2}. The mitochondrial activity of nicotinamide adenine dinucleotide (NADH) dehydrogenase in the cells in each well was measured at 18 h after photoirradiation using Alamar Blue reagent (10 μL, DAL1025, Biosource, Camarillo, CA, USA) according to the manufacturer's instructions. The absorbance at 570 nm was measured using a plate reader. The percentage cell survival was calculated by normalization with respect to a control experiment containing no drug.

Acknowledgements

We gratefully acknowledge Prof. Yuzo Fujii for his valuable suggestions and discussions. This work was performed within the framework of the KOSEN-GIKADAI project, and partly sponsored by NIT, Yonago College financial support.

References

1. M, Tim. *Mini-Rev. Med. Chem.*, **2009**, *9*, 974.
2. R. Bonnett. *Chem. Soc. Rev*. **1995**, *24*, 19.
3. Lang, K.; Mosinger, J.; Wagnerovia, D. M. *Coord. Chem. Rev.*, **2004**, *248*, 321.
4. Mikata, Y.; Takagi, S.; Tanahashi, M.; Ishii, S.; Obata, M.; Miyamoto, Y.; Wakita, K.; Nishisaka, T.; Hirano, T.; Ito, T.; Hoshino, M.; Ohtsuki, C.; Tanihara, M.; Yano, S. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3289.
5. Warburg, O. *Science*, **1956**, *123*, 309.
6. Sol, V.; Blais, J. C.; Bolbach, G.; Carre, V.; Granet, R.; Guilloton, M.; Spiro, M.; Krausz, P. *Tetrahedron Lett.*, **1997**, *38*, 6391.
7. Maillard, P.; Hery, C.; Momenteau, M. *Tetrahedron Lett.*, **1997**, *38*, 3731.
8. Oulmi, D.; Maillard, P.; Guerquin-Kern, J-L.; Huel, C.; Momenteau, M. *J. Org.*

Chem., **1995**, *60*, 1554.

9. Hansen, J. B.; Bjerring, P.; Buchardt, O.; Ebbesen, P.; Kanstup, A.; Karup, G.; Knudsen, P. H.; Nielsen, P. E.; Norden, B.; Ygge, B. *J. Med. Chem.*, **1985**, *28*, 1001.
10. Mereyala, H. B.; Gurrala, S. R. *Carbohydr. Res.*, **1998**, *307*, 351.
11. Aboulezz, F.; EI-Attar, A. A.; EI-Sockary, M. A. *Acta chimica Academiae Scientiarum Hungaricae* **1973**, *77*, 205.
12. Hartmuth, C.; kolb, M.; G. Finn, and K. Barry Sharpless, *Angew. Chem. Int. Ed.* **2001**, *40*, 2004.
13. Spiller, W.; Kliesch, H.; Wöhrle, D.; Hackbarth, S.; Röder, B.; Schnurpfeil, G. *J. Porphyrins Phthalocyanines* **1998**, *2*, 145.
14. Kuchta, K.; Ortwein, J.; L. Hennig, HW. *Rauwald, Fitoterapia* **2014**, *96*, 8.
15. Hirohara, S.; Obata, M.; Alitomo, H.; Sharyo, K.; Ando, T.; Tanihara, M.; Yano, S. *J. Photochem. Photobiol*. B: Biol. **2009**, *97*, 22.
16. Obata, M.; Hirohara, S.; Sharyo, K.; Alitomo, H.; Kajiwara, K.; Ogata, S.; Tanihara, M.; Ohtsuki, C.; Yano, S. *Biochim. Biophys. Acta*. **2007**, *1770*, 1204.
17. Hansen, J. B.; Buchardt, O. *Tetrahedron Lett.* **1981**, *22*, 1847.