Target-selective degradation of proteins by a light-activated 2-phenylquinoline-estradiol hybrid†

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A designed 2-phenylquinoline-estradiol hybrid effectively and selectively degraded the target transcription factor, human estrogen receptor-α (hER-α), which has a high affinity with the estradiol moiety, under long-wavelength UV photo-irradiation, without additives and under neutral conditions.

Proteins are key players in many biological events. The development of new methods for selective control of specific protein functions is of considerable importance in the fields of chemistry, biology, and medicine. In this context, the possibility of developing an organic photochemical agent that can degrade proteins by irradiation with a specific wavelength of light under mild conditions and without any additives (such as metals or reducing agents) has attracted much attention.[1] We reported recently that anthraquinones can act as protein photocleavers.[2] However, there have as yet been no reports of methods in which a light-activated agent of this type is used for selective degradation of a target protein. Here, we report the target-selective degradation of a protein induced by a light-activated small organic molecule. A 2-phenylquinoline derivative was found to be capable of degrading proteins under long-wavelength UV photo-irradiation, without additives and under neutral conditions. Furthermore, the designed and synthesized 2-phenylquinoline-estradiol hybrid effectively and selectively degraded the target protein, a transcription factor known as human estrogen receptor-α (hER-α), which has a high affinity with the estradiol moiety. To the best of our knowledge, this is the first successful example of target-selective degradation of a protein by light switching under neutral conditions. We anticipate that the present method will be used as a "smart" technology for selective control of specific functions of target proteins; in addition, it should prove useful for structure–activity studies of proteins, investigation of structural domains, and design of novel therapeutic drugs targeting proteins.

In our previous work, certain 2-phenylquinoline derivatives were found to be efficient agents for DNA photocleavage.[3] Based on these findings, we expected that if a 2-phenylquinoline derivative could be made to produce a radical or a reactive oxygen species (ROS) by photo-excitation, this could be used for degradation not only of DNA, but also of protein molecules. To investigate this hypothesis, we selected 2-phenylquinoline itself (**1**) as a protein photo-degrading agent, and hER-α as the target protein (Fig. 1). It has previously been reported that the 2-phenylquinoline scaffold has some similarity to estrogen in terms of its affinity with hER.[4] Furthermore, modulation of hER-α function is an important factor in a variety of diseases, including breast cancer and osteoporosis.[5]

First, we examined the photo-induced protein-degrading activity of **1** at concentrations of 10, 3.0, 1.0 and 0.1 μM against 1.0 μM of hER-α in 20% acetonitrile–Tris-HCl buffer (pH 8.0, 50 mM) using a long-wavelength UV lamp (365 nm, 100 W, 4 mW cm^{-2}) for photo-irradiation. The progress of the photo-degradation reaction was monitored by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE);[6] the results are shown in Fig. 2. Comparison of lanes 3 and 4 with lane 2 shows that neither photo-irradiation of hER-α in the absence of **1** (lane 3) or treatment of hER-α with **1** without photo-irradiation (lane 4) resulted in a change in the SDS-PAGE profile. In contrast, lane 5 shows fading of the SDS-PAGE band corresponding to hER-α after exposure to **1** with photo-irradiation, which indicates that degradation of hER-α took place. These results show that 2-phenylquinoline (**1**) is capable of degrading a protein, hER-α, upon irradiation with long wavelength UV light and without further additives, although its ability is not particularly high. Because degradation of hER-α by **1** did not take place in the absence of light, it was confirmed that UV light functioned as a trigger to initiate protein degradation by **1**. In addition, since the pattern obtained for hER-α degradation by **1** contained faded and smear bands, it was concluded that degradation of hER-α by **1** took place in a random fashion.[7]

In order to improve the protein degrading ability and selectivity of 2-phenylquinoline (**1**), we designed and synthesized hybrid molecule **2**, which consists of 2-phenylquinoline and estradiol (Fig. 1). Estradiol has very strong and selective affinity with hER-α. The hybrid **2** was synthesized by a modified version of the procedure reported by Jones *et al.* in which an enediyne was employed as a protein cleaver.[8] In the chemical synthesis of hybrid **2**, which is outlined in Scheme 1, the commercially available

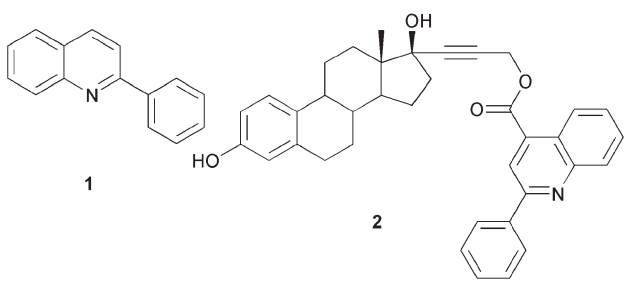

Fig. 1 Chemical structures of 2-phenylquinoline (**1**) and 2-phenylquinoline-estradiol hybrid **2**.

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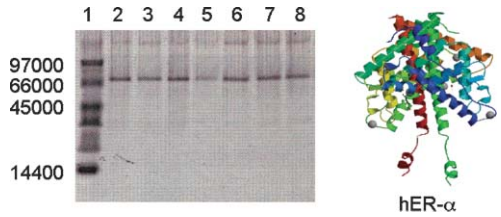
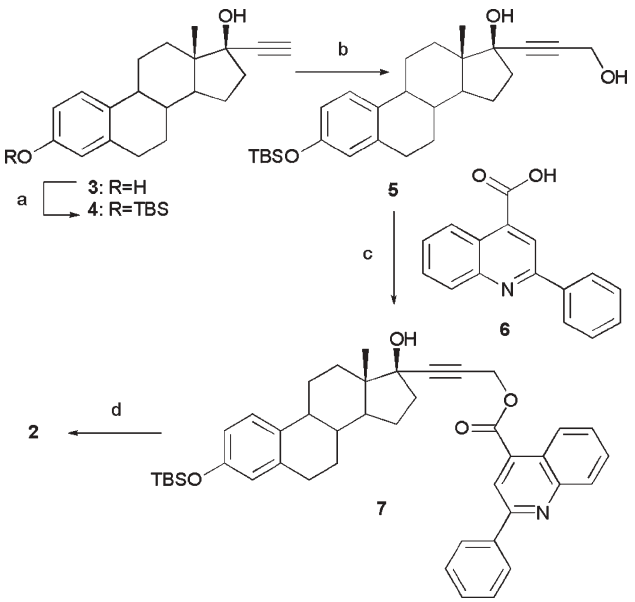


Fig. 2 Photo-degradation of human estrogen receptor-α (hER-α) using **1**. hER-α (1.0 μM) was incubated with **1** in 20% acetonitrile–Tris-HCl buffer (pH 8.0, 50 mM) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed at 10 cm from the sample, and analyzed using 8% tricine-SDS-PAGE. Lane 1, size marker; lane 2, hER-α alone; lane 3, hER-α with UV; lane 4, hER-α + **1** (10 μM) without UV; lanes 5–8, hER-α + **1** (concentrations 10, 3, 1, and 0.3 μM, respectively) with UV.



Scheme 1 Synthesis of 2-phenylquinoline-lectin hybrid **2**. (a) TBSOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to rt, 2 h, 100%; (b) LDA, $(CH_2O)_n$, THF, −78 to 0 °C, 17 h, 77%; (c) **6**, EDC, DMAP, CH_2Cl_2, 0 °C to rt, 2 h, 95%; (d) TBAF, AcOH, THF, 0 °C to rt, 1 h, 100%.

estradiol derivative **3** was regioselectively protected with a *tert*-butyldimethylsilyl (TBS) group to give the silylated compound **4**, which was then treated with lithium diisopropylamide (LDA), followed by addition of $(CH_2O)_n$ to furnish the primary alcohol **5**. Subsequent esterification of **5** with 2-phenylquinoline-4-carboxylic acid (**6**) in the presence of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and *N*,*N*-4-dimethylaminopyridine (DMAP) gave the hybrid **7**. Finally, deprotection of the TBS group in **7** using tetra-*n*-butylammonium fluoride (TBAF) and AcOH gave the desired hybrid **2**.

We then examined the application of our designed 2-phenylquinoline-estradiol hybrid **2** in target-selective photo-degradation of proteins. Photo-induced degradation of three types of protein—hER-α, bovine serum albumin (BSA) and hen egg lysozyme (Lyso)—was carried out using **2**, and the reaction progress was monitored by SDS-PAGE. The results are summarized in Fig. 3a–c. When the hybrid **2** was exposed to hER-α under photo-irradiation, significant degradation took place (Fig. 3a). The degradation ability of **2** was found to be much greater than that of

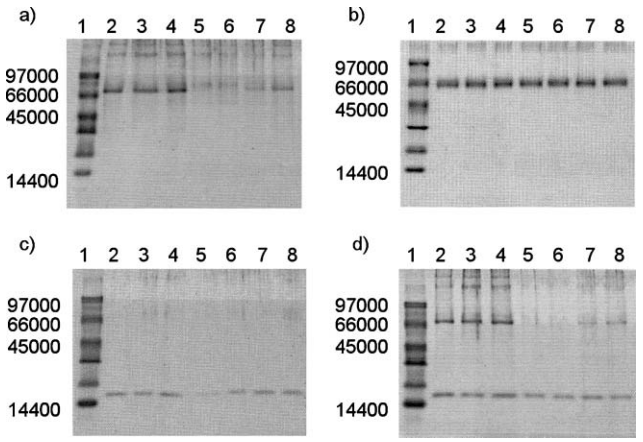


Fig. 3 Photo-degradation of proteins using **2**. Each protein (1.0 μM) was incubated with **2** in 20% acetonitrile–Tris-HCl buffer (pH 8.0, 50 mM) at 25 °C for 2 h under irradiation with a UV lamp (365 nm, 100 W) placed at 10 cm from the sample, and the products were analyzed by tricine-SDS-PAGE. (a), (b), (c), and (d) represent hER-α, BSA, Lyso, and hER-α + Lyso, respectively: lane 1, size marker; lane 2, protein alone; lane 3, protein with UV; lane 4, protein + **2** (10 μM) without UV; lanes 5–8, protein + **2** (concentrations 10, 3, 1, and 0.3 μM, respectively) with UV.

1. This result was in sharp contrast to those obtained using the other proteins (BSA and Lyso), which showed no degradation and slight degradation, respectively, under photo-irradiation with **2** (Fig. 3b and c). Furthermore, it was noteworthy that when hER-α and Lyso were both present in the reaction mixture, only hER-α was degraded by **2**, as shown in Fig. 3d. These results clearly indicate that the 2-phenylquinoline-estradiol hybrid **2** causes selective degradation only of the target protein, hER-α, upon photo-irradiation, without any additives and under neutral conditions.

The hER-α-degrading activity of the 2-phenylquinoline-estradiol hybrid **2** was found to decrease significantly in the presence of estradiol derivative **3**, which also shows strong affinity with hER-α. This result indicates that the strong and selective photo-degradation ability of **2** against hER-α depends on the high recognition ability of the estradiol moiety for hER-α. In addition, the activity of **2** decreased in the presence of $O_2^{\bullet -}$ and H_2O_2 scavengers Tiron and KI, though they are not specific for $O_2^{\bullet -}$ and H_2O_2. Furthermore, photo-irradiation of **2** in the presence of the spin trap 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) gave products with ESR spectrum characteristics of the DMPO–superoxide anion spin adduct DMPO–$^{\bullet}$OOH and the DMPO–hydroxyl radical spin adduct DMPO–$^{\bullet}$OH, which result from the reaction of DMPO with $O_2^{\bullet -}$ and the reaction of DMPO with $^{\bullet}$OH and/or the decay of DMPO–$^{\bullet}$OOH, respectively, as shown in Fig. 4.[9] It was confirmed that no peaks corresponding to DMPO–$^{\bullet}$OOH and DMPO–$^{\bullet}$OH were detected either by treatment of DMPO with **2** without photo-irradiation or by photo-irradiation of DMPO in the absence of **2** (see: ESI Fig. S1†). Although a singlet oxygen-mediated degradation mechanism could not be ruled out, it was found that the degradation ability of **2** in D_2O, in which the lifetime of singlet oxygen is extended, was similar to that in H_2O. Therefore, protein hER-α degradation must be due to reactive oxygen species (ROS) produced by photo-excitation of 2-phenylquinoline and O_2.[10]

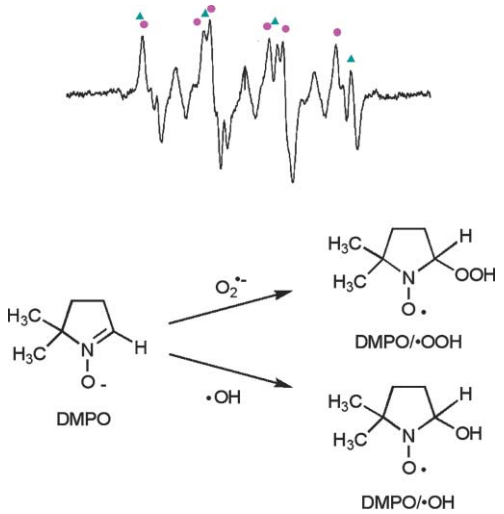


Fig. 4 ESR spectrum obtained during photo-irradiation of **2** in the presence of DMPO. Compound **2** (4 mM) and DMPO (100 mM) were used in 50% acetonitrile–Tris-HCl buffer (pH 8.0, 50 mM). The mauve circles and the green triangles represent the positions of the DMPO–˙OOH and DMPO–˙OH signals, respectively.

In conclusion, we have developed a new method for selective degradation of a target protein by photo-irradiation using a 2-phenylquinoline-estradiol hybrid under neutral conditions. The results presented here will contribute to the molecular design of novel artificial protein photo-degradation agents. We hope that this method will provide a means of controlling the specific functions of certain proteins.

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Notes and references

1 R. Miyake, J. T. Owens, D. Xu, W. M. Jackson and C. F. Meares, *J. Am. Chem. Soc.*, 1999, **121**, 7453; G. Plourde, II, A. El-Shafey, F. S. Fouad, A. S. Purohit and G. B. Jones, *Bioorg. Med. Chem. Lett.*, 2002, **12**, 2985; F. S. Fouad, J. M. Wright, G. Plourde, II, A. D. Purohit, J. K. Wyatt, A. El-Shafey, G. Hynd, C. F. Crasto, Y. Lin and G. B. Jones, *J. Org. Chem.*, 2005, **70**, 9789.
2 A. Suzuki, M. Hasegawa, M. Ishii, S. Matsumura and K. Toshima, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4624.
3 K. Toshima, R. Takano, Y. Maeda, M. Suzuki, A. Asai and S. Matsumura, *Angew. Chem., Int. Ed.*, 1999, **38**, 3733.
4 A. T. Vu, S. T. Cohn, E. S. Manas, H. A. Harris and R. E. Mewshaw, *Bioorg. Med. Chem. Lett.*, 2005, **15**, 4520.
5 J. A. Scott and W. L. McGuire, in *Endocrine-Dependent Tumors*, ed. K. D. Voight and C. Knabbe, Raven, New York, 1991, pp. 179.
6 H. Schägger and G. von Jagow, *Anal. Biochem.*, 1987, **166**, 368.
7 The aggregation of the protein was not observed at the top of the gels. Furthermore, no peaks corresponding to degraded peptide fragments were detected by MALDI-TOF MS analysis. This suggests that the degradation reaction occurred non-site-specifically and hER-α was degraded into peptide fragments that were too small to allow for SDS-PAGE or MALDI-TOF MS analysis. Also see: ref. 2; G. B. Jones, J. M. Wright, G. Hynd, J. K. Wyatt, M. Yancisin and M. A. Brown, *Org. Lett.*, 2000, **2**, 1863; T. Furuta, M. Sakai, H. Hayashi, T. Asakawa, F. Kataoka, S. Fujii, T. Suzuki, Y. Suzuki, K. Tanaka, N. Fishkin and K. Nakanishi, *Chem. Commun.*, 2005, 4575.
8 G. B. Jones, G. Hynd, J. M. Wright, A. Purohit, G. W. Plourde, II, R. S. Huber, J. E. Mathews, A. Li, M. W. Kilgore, G. J. Bubley, M. Yancisin and M. A. Brown, *J. Org. Chem.*, 2001, **66**, 3688.
9 E. Wertz and B. Bolton, *Electron Spin Resonance*, McGraw-Hill, New York, 1972; H. M. Swartz, J. R. Bolton and D. C. Borg, *Biological Application of Electron Spin Resonance*, Wiley-Interscience, New York, 1972.
10 B. Halliwell and J. M. C. Gutteridge, *Free Radicals in Biology and Medicine*, Oxford University Press, Oxford, 1985; K. J. A. Davies, *J. Biol. Chem.*, 1987, **262**, 9895.

