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Enhanced Efficacy of Gefitinib in Drug-sensitive and Drug-resistant Cancer Cell Lines after Arming with a Singlet Oxygen Releasing Moiety

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Abstract: Attractive results have been achieved with small molecular target-based anticancer drugs in the anticancer field; however, enhancing their treatment effect and solving the drug-resistant problem remain key concerns worldwide. Inspired by the specific affinity of gefitinib for tumour cells and the strong oxidation capacity of singlet oxygen, we combined a chemically generated singlet oxygen moiety with the small molecular-targeted drug gefitinib to improve its anticancer effect. We designed and synthesised a novel compound (**Y5-1**), in which a small molecular targeted therapy agent (gefitinib) and a singlet oxygen (provided by an in vitro photodynamic reaction) thermal-controlled releasing moiety are covalently conjugated. We demonstrated that the introduction of the singlet oxygen thermal-controlled releasing moiety enhanced the anticancer activities of gefitinib. The results of this study are expected to provide a novel strategy to enhance the effect of chemotherapy drugs on drug-resistant cell lines.

Introduction

Cancer is the second leading cause of death and has become a huge global health burden.[1] The discovery and development of novel and efficient anticancer drugs or strategies for cancer treatment are the most important goals in modern medicinal chemistry.[2] In clinical trials, small-molecule inhibitors of tyrosine kinase (TKI) represent a major advance in cancer treatment.[3] The first rationally designed small-molecule inhibitor, imatinib (Gleevec®, Novartis), was approved by the FDA for the therapy of chronic myeloid leukaemia (CML) in 2001, and this development is considered to have started a new era in anticancer drug discovery.[4] Subsequently, more than 30 small molecular-targeted drugs have been approved for clinical application to treat different types of human cancers.[5] Gefitinib, a small molecular-targeted anticancer drug, can target the ATP-binding domain of tyrosine kinase in epidermal growth factor receptor (EGFR)-overexpressing tumours and has exhibited specific affinity for tumour cells.[6] However, accumulating clinical evidence suggests an unsatisfactory treatment effect and drug resistance.[7]

Much effort has been devoted to developing novel small molecular-targeted anticancer drugs or other new strategies to improve the anticancer treatment effect of gefitinib and overcome resistance.[8] One such strategy is to design new-generation TKIs such as afatinib and avitinib;[9] unfortunately, most of them have been found to have poor clinical patient outcomes or serious side effects such as hyperglycaemia.[10] Another approach is the combination of gefitinib with other drugs such as docetaxel, bisphosphonate zoledronic acid, and chloroquine diphosphate;[11] however, this approach is difficult to apply in clinical practice and may result in more side effects. Another method is to use a nanocarrier for gefitinib and other anticancer drugs; for example, functionalised graphene oxide has been used as a nanocarrier for gefitinib and quercetin.[12] However, the dosage of anticancer drugs is not accurate.

Photosensitisers are considered controllable and safe anticancer drugs.[13] During photodynamic therapy, singlet oxygen, which is produced by energy transfer from an excited photosensitiser to oxygen, can induce oxidative damage to cellular components (e.g. proteins, lipids, and DNA), apoptosis, or necrosis.[14] For several years, our research group has designed and synthesised many novel anticancer drugs.[15] We have conjugated small molecular-targeted drugs such as gefitinib with a phthalocyanine core and demonstrated that these conjugates are highly promising antitumor agents.[15] However, these agents face several challenges, including therapy dosimetry and reliance on oxygen, light, and PSs.[16] Based on the specific affinity of gefitinib for tumour cells and the strong oxidation capacity of singlet oxygen, we hypothesised that combining a chemical source of singlet oxygen with gefitinib in a single molecule would improve the anticancer effect of gefitinib. Introducing chemically generated singlet oxygen into small molecular-targeted drugs would be of great interest and possible clinical value.

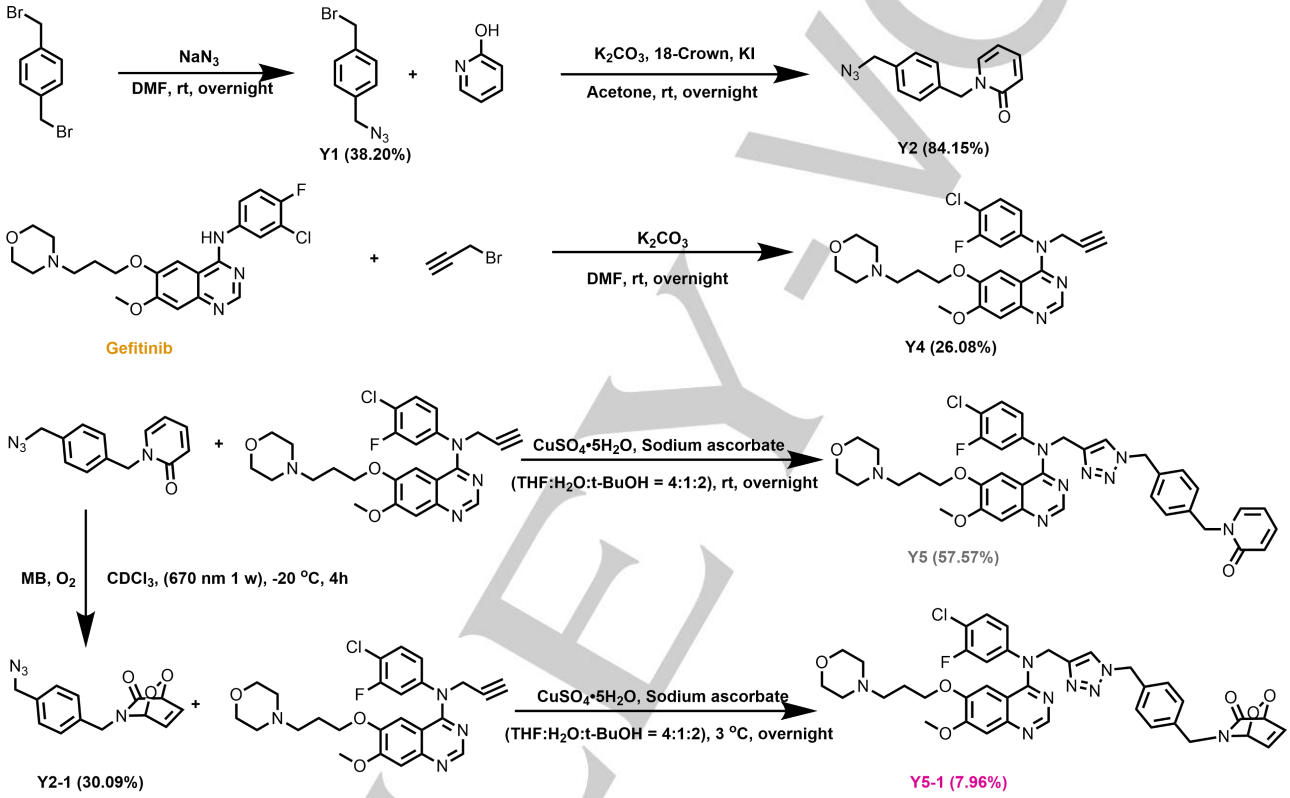
A perfect source of $^1O_2$ should have outstanding singlet oxygen yields and release singlet oxygen under mild conditions. The endoperoxides of 2-pyridone and its derivatives have been shown to have outstanding singlet oxygen yields and release singlet oxygen under body temperature.[17] They can induce the apoptosis of cancer cells by the thermal release of $^1O_2$.[17,18]

Moreover, endoperoxides can be formed by the reaction between $^1O_2$ and 2-pyridone.[17,18]

Inspired by the considerable efforts to develop more effective and safer anticancer drugs, we designed and synthesised a novel compound (**Y5-1**), in which 2-pyridone endoperoxides as a $^1O_2$ provider was conjugated with gefitinib units by a click reaction. **Y5-1** displays high affinity for and anticancer activities against cancer cells overexpressing EGFR. The results of this study may lead to improvements in the therapeutic efficacy of not only small molecular-targeted anticancer drugs but also chemotherapeutic drugs in MDR cancer cells. Based on its accurate therapy dosimetry, high selectivity, and anticancer activities, **Y5-1** may be applied in clinical practice.

Results and Discussion

The synthesis of compounds **Y5-1** and **Y5** is shown in Scheme 1. First, **Y1**, **Y2**, and **Y2-1** were synthesised by a previously published method.[19] **Y4** was synthesised by a nucleophilic reaction between gefitinib and bromopropyne. Subsequently, **Y5-1** was synthesised by the click reaction of **Y2-1** and **Y4** with $CuSO_4{\cdot}5H_2O$ and L-ascorbic acid sodium salt as catalysts. **Y5** was synthesised in a simple and similar manner.



Scheme 1. Synthesis of **Y5-1** and **Y5**.

An $^1O_2$ scavenger (1,3-diphenylisobenzofuran, DPBF) was used to test the $^1O_2$-releasing capacity of **Y5-1**. As shown in Figure 1, the absorbance intensity of DPBF (**Y5-1** and the DPBF group) decreased at body temperature (37 °C), while the downtrend in the blank contrast and **Y5** contrast was negligible, demonstrating that the endoperoxide structure of **Y5-1** can release $^1O_2$ at 37 °C (Figure 1a). In addition, the **Y5-1** contrast showed a slight downtrend of DPBF at 25 °C (Figure 1b), demonstrating that the $^1O_2$-releasing capacity of **Y5-1** greatly decreases at 25 °C. These results indicate that **Y5-1** can slowly release $^1O_2$ at body temperature (37 °C).

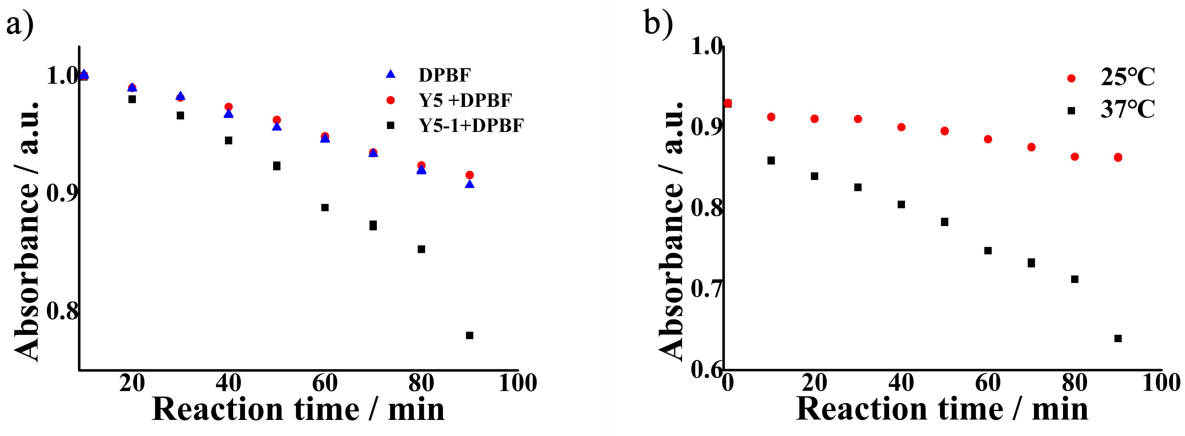


Figure 1. a) Spectrum changes of DPBF at 415 nm during the determination of singlet oxygen for **Y5** and **Y5-1** at 37 °C; b) Spectrum changes of DPBF and **Y5-1** at 415 nm vs time (25 °C and 37 °C); inset: DPBF, **Y5** (1.5 mmol), and **Y5-1** (1.5 mmol) were all dissolved in DMF.

The anticancer effects of **Y5-1**, **Y5,** and gefitinib were evaluated using the methyl thiazolyl tetrazolium (MTT) assay, in which three cancer cell lines, A549 cells (high EGFR expression), HepG2 cells (high differentiation and proliferation), and H1975 cells (gefitinib-resistant) were used. The dose-dependent survival curves are shown in Figure 2, and the corresponding IC_{50} values are listed in Table 1. The significant differences in the anticancer effects of **Y5** (with an IC_{50} = 51.3-94.2 μM) and **Y5-1** (IC_{50} = 9.2-14.8 μM) on these three cancer cell lines, demonstrating that conjugating gefitinib with endoperoxide clearly enhanced its anticancer effects. As expected, **Y5-1**, **Y5** armed with $^1O_2$, had 2.6-4.2-fold higher cytotoxicity than gefitinib. These results clearly showed that introduction of an $^1O_2$-releasing moiety into the gefitinib structure significantly enhanced its anticancer effects, and **Y5-1** had the highest cytotoxicity in gefitinib-resistant cancer cells.

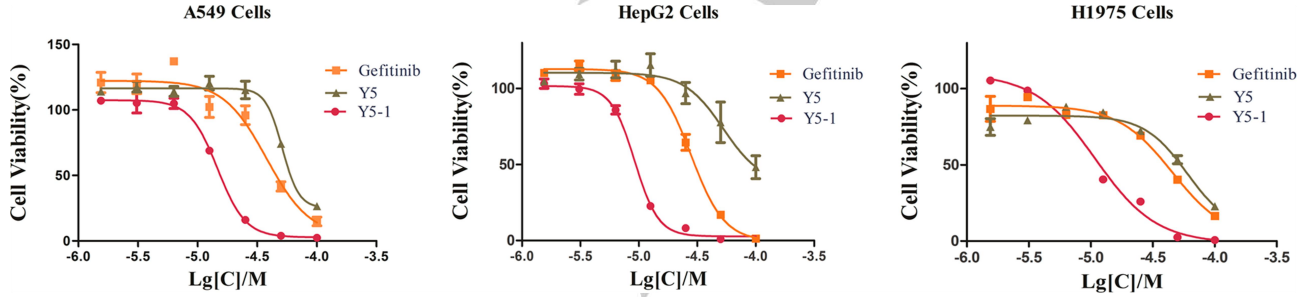


Figure 2. Cell toxicity of gefitinib (orange), **Y5** (grey), and **Y5-1** (red) on A549, HepG2, and H1975 cells (data are expressed as the mean value ± standard error of the mean value of four independent experiments).

Table 1. Cellular antiproliferative activities towards different cancer cells.

compounds	Cellular Antiproliferative Activity (IC_{50}, μM)		
	A549	HepG2	H1975
Gefitinib	38.0	28.0	45.6
Y5	51.3	94.2	60.1
Y5-1	14.8	9.2	10.8

The antiproliferative activity of **Y5-1** towards cancer cells is associated with not only cellular uptake but also singlet oxygen production. To further explain the differences in the anticancer effects of **Y5-1** against these three cell lines, cellular uptake and singlet oxygen generation efficiency were evaluated in detail. First, absorption and fluorescence spectroscopy were used to study the cellular uptake of **Y5-1**. The average uptake data are shown in Figure 3a. The uptake of **Y5-1** in A549 cells was significantly higher than that in HELF cells, HepG2 cells, and H1975 cells. These results indicate that the introduction of the gefitinib moiety into **Y5-1** imparted affinity for cancer cells with high EGFR expression and higher sensitivity to gefitinib. Second, DCFH-DA (2',7''-dichlorodihydrofluorescein diacetate) was used to measure ROS generation because the singlet

oxygen produced by **Y5-1** is a type of ROS. Moreover, the ROS level is an important parameter that reflects the anti-tumour effect. Therefore, we selected DCFH-DA to detect the production of ROS by **Y5-1**. As shown in Figure S1 (Supporting Information), for both HepG2 cells and A549 cells, the ROS generation in the **Y5-1** group was positively correlated with the concentration of **Y5-1**, and there was no significant difference in the ROS production efficiency of **Y5-1** between these two cell lines at the same concentrations. Compared with **Y5** and gefitinib, **Y5-1** can release significantly more ROS in HeLa cells (Figure 3b). Therefore, the higher cellular uptake and ROS generation of **Y5-1** make it an excellent anticancer drug.

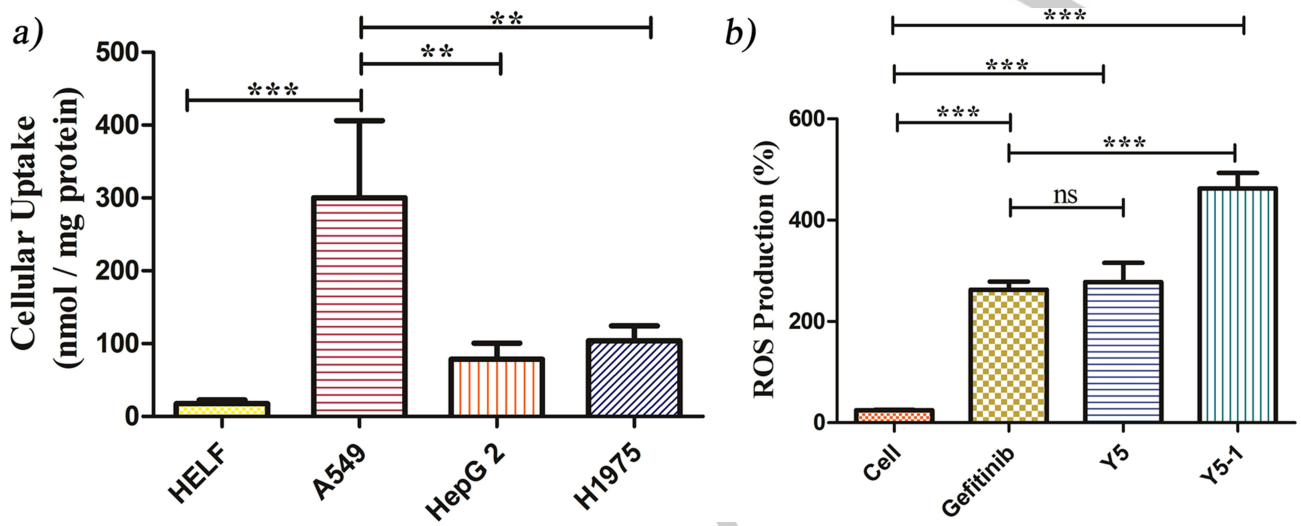


Figure 3. a) Uptake of **Y5-1** in different cell lines. b) Generation of ROS in HeLa cells with no addition, gefitinib (25 μM), **Y5** (25 μM), and **Y5-1** (25 μM) (there were six independent experiments for each group; statistical analysis: ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$, ns indicates $p > 0.05$).

Conclusion

We designed and synthesised a novel compound (**Y5-1**) and evaluated its in vitro anticancer activities. This compound contains a small molecular-targeted agent (gefitinib) and a singlet oxygen (added in an in vitro photodynamic reaction) thermal-controlled releasing moiety, which are covalently linked and act in a cooperative manner. We demonstrated that **Y5-1** exhibits high specific affinity for EGFR-overexpressing cancer cells. Additionally, the introduction of the $^1O_2$ thermal-controlled releasing moiety clearly enhanced the anticancer activities of gefitinib in drug-resistant cell lines. To enable the use of **Y5-1** for clinical applications, more detailed studies on its anti-tumour mechanisms and in vivo animal experiments are in progress. Overall, this work presents not only a novel compound with efficient anticancer activity but also a design strategy for developing high-efficiency anticancer drugs.

Experimental Section

All reagents were purchased from commercial sources. ^{13}C NMR and ^{1}H NMR spectra were recorded on a nuclear magnetic resonance device with AVANCE III (400 MHz) or AVANCE III 500 (500 MHz). DMSO-d_6 and $CDCl_3$ were used as deuterated reagents. HRMS data were obtained using LC-QTOF-MS (G6520B). The product purity was greater than 95%. A Hitachi 4510-UV-Visible Absorption Spectrometer was used to detect the $^1O_2$ released by **Y5-1**. The anticancer activity of **Y5-1** was evaluated from three aspects, including cytotoxicity, cellular uptake, and ROS production ability. The remaining instruments included a spectraMax@ i5x.

Synthesis of Compound Y4. Gefitinib and propargyl bromide were dissolved in N,N-dimethylformamide (10 mL) and stirred at 37 °C for 25 h. The product was purified by column chromatography (CH_3OH/CH_2Cl_2 from 1:100 to 1:40). Compound **Y4** was obtained as a yellow solid (0.3087 g, 26.08%). ^{1}H NMR (400 MHz, DMSO-d6) δ 8.72 (s, 1H), 7.64 (dd, J = 6.3, 2.3 Hz, 1H), 7.47 (t, J =9.0 Hz, 1H), 7.22 (d, J = 3.8 Hz, 2H), 6.38 (s, 1H), 4.85 (s, 2H), 3.90 (s, 3H), 3.60 – 3.55(m, 4H), 3.51 (t, J = 6.5 Hz, 2H), 3.15 (s, 1H), 2.30 (d, J = 7.9 Hz, 4H), 1.69 (p, J = 6.4 Hz, 2H), 1.31–1.21 (m, 2H). ^{13}C NMR (126 MHz, DMSO-d6) δ 159.14, 154.63, 152.94, 149.32, 147.41, 143.39, 128.56, 127.51, 127.45, 118.29, 118.12, 110.51, 107.82, 105.16, 80.35, 75.41, 66.61, 66.56, 56.35, 55.37, 55.01, 53.80, 42.55, 25.62. HRMS (ESI) m/z calcd $C_{25}H_{26}ClFN_4O_3$ $[M+H]^+$: 484.1677, found : 485.1756.

Synthesis of Compound Y5. Compound **Y2** (0.1655 g, 0.689 mmol), **Y4** (0.3146 g, 0.649 mmol), sodium ascorbate (0.0202 g, 0.1018 mmol), and $CuSO_4{\cdot}5H_2O$ (0.0253 g, 0.1012 mmol) were dissolved in a mixture reagent (tBu-OH/H_2O/THF, 4:2:8 mL). The mixture was stirred for 23 h at 37 °C. The product was purified by column chromatography (CH_3OH/CH_2Cl_2 from 1:100 to 1:20) to acquire **Y5** (0.2582 g, 57.57%) as a light-yellow solid. ^{1}H NMR (400 MHz, DMSO-d6) δ 8.68 (s, 1H), 8.16 (s, 1H), 7.73 (d, J = 6.5 Hz, 1H),7.55 (d, J = 6.4 Hz, 1H), 7.39 (q, J = 9.1, 7.9 Hz, 2H), 7.25 - 7.17 (m, 4H), 7.14 (d, J =7.8 Hz, 2H), 6.43–6.35 (m, 2H), 6.21 (t, J = 6.7 Hz, 1H), 5.49 (s, 2H), 5.33 (s, 2H), 5.05(s, 2H), 3.90 (s, 3H), 3.58 (s, 4H), 3.47 (t, J = 6.4 Hz, 2H), 2.32 (s, 4H), 1.73–1.63 (m, 2H), 1.23 (s, 2H).^{13}C NMR (101 MHz, DMSO-d6) δ 161.81, 159.30, 154.51, 153.00, 147.20, 143.75,140.51, 139.50, 137.68, 135.93, 128.60, 128.41, 128.22, 127.44, 127.37, 125.00, 120.33, 118.21, 118.00, 107.87, 105.97, 105.47, 66.56, 66.54, 56.34, 54.99, 53.76,52.81, 51.20, 48.53, 25.62. HRMS (ESI): calcd $C_{38}H_{38}ClFN_8O_4$ $[M+H]^+$: 725.2761, found: 725.2779, Δ=2.48 ppm.

Synthesis of Compound Y5-1. Compound **Y4** (0.3939 g, 0.8081 mmol), **Y2-1** (0.2 g, 0.7346 mmol), sodium ascorbate (0.0198 g, 0.1002 mmol), and $CuSO_4{\cdot}5H_2O$ (0.0252 g, 0.1010 mmol) were dissolved in a mixture solvent (tBu-OH/H_2O/THF, 4:2:8 mL). The solution was stirred for 24 h at 3 °C. The product was purified by column chromatography (CH_3OH/CH_2Cl_2 from 1:100 to 1:40) to acquire compound **Y5-1** (0.0478 g, 7.96%) as a white solid. ^{1}H NMR (500 MHz, chloroform-d) δ 8.60 (s, 1H), 7.50 (s, 1H), 7.34 (dd, J = 6.5, 2.4 Hz, 1H), 7.28 (s, 2H), 7.24 (s, 1H), 7.21–7.16 (m, 3H), 7.12 (s, 2H), 7.12 (s, 2H), 6.30 (s, 1H), 5.77 (d, J = 6.1 Hz, 1H), 5.56–5.52 (m, 1H), 5.47 (q, J = 4.8, 4.0 Hz, 1H), 5.41 (d, J= 9.2 Hz, 1H), 5.40–5.34 (m, 3H), 5.16 (s, 1H), 5.13 (s, 1H), 5.05 (s, 1H), 5.01 (d, J =9.3 Hz, 1H), 4.23 (dd, J = 15.1, 7.3 Hz, 1H), 4.18 – 4.14 (m, 1H), 3.96 (s, 1H), 3.93 (d, J= 3.1 Hz, 3H), 3.71 (d, J = 4.1 Hz, 1H), 2.25– 2.21 (m, 1H), 2.19 (s, 1H), 2.03 (q, J = 6.6 Hz, 1H), 1.83 (s, 3H). The compound decomposes; thus, its ^{13}C NMR data are not available. HRMS (ESI): m/z calcd $C_{40}H_{42}ClFN_8O_8$ $[M+CH_3COOH]^+$: 817.2871, found: 817.2870, Δ=0.13 ppm.

Singlet Oxygen ($^1O_2$) Release Experiments. The release of $^1O_2$ was tested using 1,3-diphenylisobenzofuran as a $^1O_2$ quencher. The mixture solutions of **Y5** and DPBF or **Y5-1** and DPBF were placed in water at 25 °C or 37 °C and kept in the dark. The absorption spectra were measured at 415 nm.

Cytotoxicity Experiment. First, 5000 A549, H1975, and HepG2 cells were seeded into each well of a 96-well plate and incubated 24 h. The deliquescent drugs (gefitinib, **Y5**, and **Y5-1**) were diluted with culture medium to different concentrations, and the drugs were then added to the plate. Every concentration had six replicates. After incubation for 24 h, pre-prepared MTT (10 μL, 5 μg/μL in H_2O) was added to the 96-well plate followed by incubation at 37 °C under 5% CO_2 for 4 h. The formazan was dissolved in dimethylsulfoxide (100 μL/well), and the pate was shaken for 30 min. Absorbance at 570 nm was measured using a SpectraMax@ i5x.

Cellular Uptake. First, 5000 HELF, A549, H1975, and HepG2 cells were seeded into a 96-well plate and incubated at 37 °C for 24 h. Each experiment was repeated six times. Then, 100 μL medium containing a drug at 25 μM was added to each well and incubated for 24 h. The plate was washed with physiological saline solution three times to remove the drugs. Then, the cells were lysed with 1% SDS solution (120 μL). The uptake of drugs was detected with a plate reader (excitation at 335 nm, emission at 481 nm). The quantity of cellular protein was measured using a BCA Protein Assay Kit.

Cellular ROS Production Assessment. ROS in cells was detected using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) as a probe because it can react with ROS and generate the fluorescent substance DCF. The cancer cells were seeded into a black 96-well plate at 5000 cells per 100 μL and incubated for 24 h. The old culture medium was removed, each well was washed with physiological saline solution, and new medium with a drug at a concentration of 25 μM was added. A control group of feminine and masculine was established. After incubation for 24 h, the black 96-well plate was washed with physiological saline solution and incubated with 40 μL of DCFH-DA (10 μM). After incubation for 30 min, the plate was washed with physiological saline solution four times, and the cells were lysed with 120 μL of 1% SDS for 30 min. The fluorescence of DCF was measured using a plate reader (excitation at 488 nm, emission at 525 nm).

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Keywords: Drug-resistant • Chemotherapy • Drug design • Singlet oxygen

[1] a) F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, *CA Cancer J. Clin*. **2018**, *68*, 394-424; b) M. K. Paul, A. K. Mukhopadhyay, *Int. J. Med. Sci*. **2004**, *1*, 101-115.

[2] a) G. L. Patrick, Oxford, UK, Oxford University Press Inc, 2013, pp. 489-553; b) S. Frantz, *Nature* **2005**, *437*, 942-943; c) J. Zugazagoitia, C. Guedes, S. Ponce, I. Ferrer, S. Molina-Pinelo, L. Paz-Ares, *Clin. Ther*. **2016**, *38*, 1551-1566; d) A. Gschwind, O. M. Fischer, A. Ullrich, *Nat. Rev. Cancer* **2004**, *4*, 361-370.

[3] a) A. Gschwind, O. M. Fischer, A. Ullrich, *Nat. Rev. Cancer*. **2004**, *4*, 361-370; b) C. Yewale, D. Baradia, I. Vhora, S. Patil, A. Misra, *Biomaterials*. **2013**, *34*, 8690-8707.

[4] N. Listed, *Med. Lett. Drugs Ther*. **2001**, *43*, 49-52.

[5] a) M. Huang, A. Shen, J. Ding, M. Geng, *Trends Pharmacol. Sci*. **2014**, *35*, 41-50; b) C. Sawyers, *Nature*. **2004**, 432, 294-297; b) B. J. Druker, *Oncologist*. **2004**, *9*, 357-360.

[6] a) K. D. Carey, A. J. Garton, M. S. Romero, J. Kahler, S. Thomson, S. Ross, F. Park, J. D. Haley, N. Gibson, M. X. Sliwkowski, *Cancer Res*. **2006**, *66*, 8163-8171; b) V. A. Pollack, D. M. Savage, D. A. Baker, K. E. Tsaparikos, D. E. Sloan, J. D. Moyer, E. G. Barbacci, L. R. Pustilnik, T. A. Smolarek, J. A. Davis, M. P. Vaidya, L. D. Arnold, J. L. Doty, K. K. Iwata, M. J. Morin, *J. Pharmacol. Exp. Ther*. **1999**, *291*, 739-748; c) J. R. Johnson, M. Cohen, R. Sridhara, Y. F. Chen, G. M. Williams, J. Duan, J. Gobburu, B. Booth, K. Benson, J. Leighton, L. S. Hsieh, N. Chidambaram, P. Zimmerman, R. Pazdur, *Clin. Cancer Res*. **2005**, *11*, 6414-6421.

[7] a) M. Wadleigh, D. J. DeAngelo, J. D. Griffin, R. M. Stone, *Blood*. **2005**, *105*, 22-30; b) M. E. Gorre, M. Mohammed, K. Ellwood, N. Hsu, R. Paquette, P. N. Rao, C. L. Sawyers, *Science*. **2001**, *293*, 876-880; c) M. A. Fabian, W. H. Biggs, D. K. Treiber, C. E. Atteridge, M. D. Azimioara, M. G. Benedetti, T. A. Carter, P. Ciceri, P. T. Edeen, M. Floyd, J. M. Ford, M. Galvin, J. L. Gerlach, R. M. Grotzfeld, S. Herrgard, D. E. Insko, M. A. Insko, A. G. Lai, J. M. Lelias, S. A. Mehta, Z. V. Milanov, A. M. Velasco, L. M. Wodicka, H. K. Patel, P. P. Zarrinkar, D. J. Lockhart, *Nat. Biotechnol*. **2005**, *23*, 329-336; d) J. Zhang, P. L. Yang, N. S. Gray, *Nat. Rev. Cancer*. **2009**, *9*, 28-39.

[8] a) H. Daub, K. Specht, A. Ullrich, Nat. Rev. *Drug Discov*. **2004**, *3*, 1001-1010; b) G. R. Oxnard, M. E. Arcila, J. Chmielecki, M. Ladanyi, V. A. Miller, W. Pao, *Clin. Cancer Res*. **2011**, *17*, 5530-5537.

[9] a) S.-H. I. Ou, Crit. *Rev. Oncol. Hemat.* **2012**, *83*, 407-421; b) A. O. Walter, R. T. T. Sjin, H. J. Haringsma, K. Ohashi, J. Sun, K. Lee, A. Dubrovskiy, M. Labenski, Z. Zhu, Z. Wang, M. Sheets, T. St Martin, R. Karp, D. van Kalken, P. Chaturvedi, D. Niu, M. Nacht, R. C. Petter, W. Westlin, K. Lin, S. Jaw-Tsai, M. Raponi, T. Van Dyke, J. Etter, Z. Weaver, W. Pao, J. Singh, A. D. Simmons, T. C. Harding, A. Allen, *Cancer Discov*. **2013**, *3*, 1404-1415; c) S. Chang, L. Zhang, S. Xu, J. Luo, X. Lu, Z. Zhang, T. Xu, Y. Liu, Z. Tu, Y. Xu, X. Ren, M. Geng, J. Ding, D. Pei, K. Ding, *J. Med. Chem*. **2012**, *55*, 2711-2723.

[10] a) F. Eskens, C. H. Mom, A. S. T. Planting, J. A. Gietema, A. Amelsberg, H. Huisman, L. van Doorn, H. Burger, P. Stopfer, J. Verweij, E. de Vries, *Brit. J. Cancer.* **2008**, *98*, 80-85; b) K.-K. Wong, *Clin. Cancer Res*. **2007**, *13*, 4593S-4596S; c) Y. Kim, J. Ko, Z. Cui, A. Abolhoda, J. S. Ahn, S.-H. Ou, M.-J. Ahn, K. Park, *Mol. Cancer Ther.* **2012**, *11*, 784-791; d) D.-W. Kim, *Ann. Oncol.* **2015**, *26*, ii14-ii14; e) L. V. Sequist, S. Jean-Charles, J. W. Goldman, H. A. Wakelee, S. M. Gadgeel, V. Andrea, P. Vassiliki, B. J. Solomon, G. R. Oxnard, D. Rafal, *N. Engl. J. Med*. **2015**, *372*, 1700-1709.

[11] a) O. Engebraaten, H. Edvardsen, E. Lokkevik, B. Naume, V. Kristensen, L. Ottestad, V. Natarajan, *ISRN oncology.* **2012**, 176789-176789; b) C. John Wen-Cheng, H. Jia-Juan, S. Yung-Chi, Y. Kun-Yun, W. Cheng-Hsu, L. Ying-Ying, H. Todd, *Cancer Lett*. **2009**, *278*, 17-26; c) N. Ono, T. Yamazaki, T. Tsukaguchi, T. Fujii, K. Sakata, A. Suda, T. Tsukuda, T. Mio, N. Ishii, O. Kondoh, Y. Aoki, *Cancer Sci*. **2013**, *104*, 1346-1352; d) A. Azzariti, J. M. Xu, L. Porcelli, A. Paradiso, *Biochem. Pharmacol.* **2004**, *68*, 135-144; e) X. Xia, Y. Liu, Y. Liao, Z. Guo, C. Huang, F. Zhang, L. Jiang, X. Wang, J. Liu, H. Huang, *Eur. J. Pharmaco*. **2019**, *856,* 172409.

[12] a) H. Tiwari, N. Karki, M. Pal, S. Basak, R. K. Verma, R. Bal, N. D. Kandpal, G. Bisht, N. G. Sahoo, *Colloids Surfaces B.* **2019**, *178*, 452-459; b) Z. Zhou, M. Jafari, V. Sriram, J. Kim, J. Y. Lee, S. J. Ruiz-Torres, S. E. Waltz, *Mol. Pharmaceut*. **2017**, *14*, 4551-4559; c) H. Peng, B. Chen, W. Huang, Y. Tang, Y. Jiang, W. Zhang, Y. Huang, *Nano. Lett.* **2017**, *17*, 7684-7690.

[13] a) D. E. J. G. J. Dolmans, D. Fukumura, R. K. Jain, *Nat. Rev. Cancer*. **2003**, *3*, 380-387; b) A. P. Castano, P. Mroz, M. R. Hamblin, *Nat. Rev. Cancer 6*, 535-545; c) Y. Z. Shen, A. J. Shuhendler, D. J. Ye, J. J. Xu, H. Y. Chen, *Chem. Soc. Rev*. **2016**, *45*, 6725-6741.

[14] J. P. Celli, B. Q. Spring, I. Rizvi, C. L. Evans, K. S. Samkoe, S. Verma, B. W. Pogue, T. Hasan, *Chem. Rev*. **2010**, *110*, 2795-2838.

[15] a) X. Jia, F.-F. Yang, J. Li, J.-Y. Liu, J.-P. Xue, *J. Med. Chem*. **2013**, *56*, 5797-5805; b) F.-L. Zhang, Q. Huang, K. Zheng, J. Li, J.-Y. Liu, J.-P. Xue, *Chem. Commun*. **2013**, *49*, 9570-9572; c) F.-L. Zhang, Q. Huang, J.-Y. Liu, M.-D. Huang, J.-P. Xue, *ChemMedChem.* **2015**, *10*, 312-320; d) J. Chen, H. Ye, M. Zhang, J. Li, J. Liu, J. Xue, *Chin. J. Chem.* **2016**, *34*, 983-988; e) J.-J. Chen, Y.-Z. Huang, M.-R. Song, Z.-H. Zhang, J.-P. Xue, *ChemMedChem.* **2017**, *12*, 1504-1511; f) X. Zhao, Y. Huang, G. Yuan, K. Zuo, Y. Huang, J. Chen, J. Li, J. Xue, *Chem. Commun*. **2019**, *55*, 866-869.

[16] a) Z. Zhou, J. Song, L. Nie, X. Chen, *Chem. Soc. Rev*. **2016**, *45*, 6597-6626; b) T. C. Zhu, M. M. Kim, X. Liang, J. C. Finlay, T. M. Busch, *Photonics Lasers Med*. **2015**, *4*, 59-71.

[17] a) C. Wiegand, E. Herdtweck, T. Bach, *Chem. Commun*. **2012**, *48*, 10195-10197; b) D. Posavec, M. Zabel, U. Bogner, G. Bernhardt, G. Knoer, *Org. Biomol. Chem*. **2012**, *10*, 7062-7069; c) S. Benz, S. Noetzli, J. S. Siegel, D. Eberli, H. J. Jessen, *J. Med. Chem*. **2013**, *56*, 10171-10182.

[18] I. S. Turan, D. Yildiz, A. Turksoy, G. Gunaydin, E. U. Akkaya, *Angew. Chem. Int. Ed*. **2016**, *55*, 2875-2878.

[19] J. Chen, D. Li, B. Huo, F. Zhang, X. Zhao, G. Yuan, D. Chen, M. Song, J. Xue, *Mol. Pharmaceut*. **2019**, *16*, 3703-3710.

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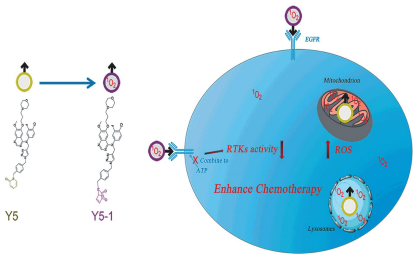

we introduce the chemically generated singlet oxygen moiety into gefitinib, gefitinib can inhibit the activity of tyrosine kinase and the $^1O_2$ thermal-controlled releasing moiety can obviously enhance the anticancer activities of gefitinib on the drug-resistant cell lines by Increasing intracellular $^1O_2$ level.

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