Journal of Materials Chemistry B

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal | View Issue

Cite this: *J. Mater. Chem. B*, 2020, **8**, 1971

Facilitating Myers–Saito cyclization through acid-triggered tautomerization for the development of maleimide-based antitumor agents†

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Received 17th November 2019,
Accepted 28th January 2020

DOI: 10.1039/c9tb02589h

rsc.li/materials-b

Enyne-allene compounds undergo Myers–Saito cyclization at physiological temperature to generate diradical intermediates that are capable of inducing DNA damage and cell death. The high reactivity of enyne-allene however limits their promising prospect as anticancer agents due to the spontaneous cyclization during storage and delivery. Regulating the cyclization process by taking advantage of the characteristics of a tumor cellular microenvironment, such as employing a low pH value to activate the cyclization process, is thus of essential importance. In this work, a novel enediyne (EDY) system with locked carbonyl groups was specifically designed and synthesized. Unlocking the protected carbonyl groups in the presence of acid would facilitate the rearrangement of propargyl moieties into an allene group, enabling the formation of an enyne-allene structure and occurrence of Myers–Saito cyclization. The pH-dependent diradical generation and DNA-cleavage ability of the designed EDY system were confirmed by electron paramagnetic resonance analysis and DNA gel electrophoresis. A promising cytotoxicity against HeLa cells with half inhibition concentrations (IC_{50}) as low as 1.40 μM was obtained, which was comparable to those of many commercially applied anticancer drugs. Further *in vitro* experiments revealed that this EDY system induced intracellular DNA damage and subsequently resulted in S-phase arrest and cytotoxicity through programmed apoptosis.

Introduction

Exploring new chemotherapeutic agents against tumor cells is of great significance because of the tremendous tragedies caused by cancer.[2–4] Many efforts have been dedicated to the synthesis of new enediyne (EDY) compounds[5–7] to develop novel anticancer drugs, as natural EDY compounds have been hailed as the most potent antitumor agents for their excellent DNA damaging ability and cytotoxicity toward tumor cells.[8] For most natural EDY compounds, the cytotoxicity originates from the cleavage of DNA by diradical intermediates generated from the EDY "warhead" through Bergman-type cyclization[9] with one exception of neocarzinostatin (NCS), which is activated by intracellular thiols and transformed to an enyne-allene structure to produce diradical intermediates through Myers–Saito cyclization.[10,11]

Since the first elucidation of the cyclization mechanism of NCS, the highly reactive enyne-allene structures have been intensively studied to show promising prospects in various fields,[12–20] including anticancer application.[21,22] The high reactivity of enyne-allene structures enables these compounds to cyclize under mild conditions, producing diradicals which are able to cause tumor cell death. However, such high reactivity of enyne-allene type compounds also limits their performance as chemotherapeutic agents, mainly due to the inevitable cyclization during storage and delivery, leading to the loss of bio-reactivity before cellular internalization and contacting DNA. Indeed, there were very few reports that focused on the bioactivities of enyne-allene compounds although the cyclization mechanism has been intensively studied.[9,10,23–26]

To develop enyne-allene compounds with potent anticancer performance, many strategies have been explored to modulate the reactivity of enyne-allene moieties with different kinds of stimuli. Schmittel[27] reported a photochemical method to activate Myers–Saito cyclization (and C^2–C^6 cyclization) of enyne-allene structures, which has potential application for photodynamic therapy. For these compounds, the thermal cyclization would only occur at elevated temperature (over 130 °C). Other promising strategies were applied through the isomerization of stable EDYs

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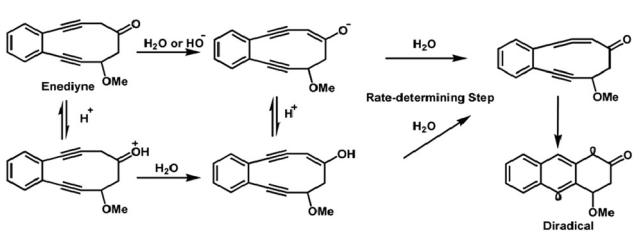
† Electronic supplementary information (ESI) available: Experimental details and spectral data. See DOI: 10.1039/c9tb02589h

to form reactive enyne-allene systems that spontaneously underwent Myers–Saito cyclization,[28] including the formation of "warhead" structures in the presence of acid, base, oxidant and transition metal complexes.[9,25,28–31]

Among these strategies, activation of the Myers–Saito reaction with acidic triggers is very promising in anticancer drug development. This method benefits from the relatively acidic microenvironment of tumor induced by the increased production of lactic acid, which can be used to differentiate tumor from normal tissues.[28,32] Moreover, it is confirmed that the pH value of tumor cells could be further lowered with hyperglycemic agents, while normal cells remain unaffected.[33] Besides the extracellular acidic environment of the tumor (pH ≈ 6.4–6.8), more acidic intracellular vesicles, such as endosomes (pH ≈ 5.5–6.0) and lysosomes (pH ≈ 4.5–5.0), provide advantages for the better design of acid-sensitive anticancer drugs.[34,35] Shibuya *et al.*[28,36,37] demonstrated a series of interesting work applying this kind of strategy, in which elaborately designed EDY compounds were transformed into reactive enyne-allene and generated diradical intermediates through Myers–Saito cyclization in acidic or basic media.

In a more realistic prospect, the acid/base regulated formation of reactive enyne-allene should occur at the pH window necessary for targeting hypoxic cancer cells. The development of novel EDY systems that could be transformed to enyne-allene and subsequently induce cytotoxicity at acidic pH (4.5–6.5) in tumor cells however still constitutes great challenge. To this end, one can take advantage of the *in situ* propargyl-allene tautomerization[38–42] that has long been practiced to construct reactive allene systems[43] undergoing various reactions to produce cyclic compounds.[44–49] This process is typically facilitated in the presence of bases or metal catalysts.[50] Nevertheless, for β-keto acetylene compounds, spontaneous rearrangement takes place even when no base or metal is involved.[25,51] Popik *et al.*[1] showed that cyclic EDY compounds with β-keto acetylene moieties underwent propargyl-allene rearrangement at acidic or basic pH. The whole procedure was accomplished *via* two steps: the spontaneous keto–enol tautomerization followed by the propargyl-allene rearrangement where H_2O served as the base (Scheme 1). In this work, it was also demonstrated that the overall cycloaromatization rate of the β-keto EDYs relied on the isomerization rate of β-keto acetylene, since the succeeding Myers–Saito cyclization of enyne-allene was much faster. This suggests that the molecular design strategy can be altered into seeking effective methods to control the formation of β-carbonyl EDY at intracellular pH, which can be accomplished by using carbonyl-protecting groups as the locking devices. Carbonyl-protecting groups, such as ketal, acetal or orthoester, have been widely applied for fabricating pH-sensitive materials, especially for tumor-targeting drug delivery systems,[52–57] fitting well with the demands for novel EDY systems that are able to build up enyne-allene structures at the pH window of tumor cells.

Herein, we report a series of pH-sensitive EDY compounds with protected β-keto groups. These compounds are stable at ambient temperature under neutral or basic conditions, and prone to hydrolysis under mild acidic conditions to form β-carbonyl EDY systems which further undergo spontaneous propargyl-allene tautomerization, facilitating the generation of diradical species through Myers–Saito cyclization. Computational calculations and hydrolysis study on model compounds were performed to investigate the acid-induced formation of enyne-allene structures. The pH-dependent radical generation and DNA-cleaving ability were investigated through electron paramagnetic resonance (EPR) analysis and gel electrophoresis. In addition, *in vitro* examinations revealed that these EDY compounds induced intracellular DNA damage and exhibited cytotoxicity comparable to many clinically used antitumor agents.



Scheme 1 H_2O assisted propargyl-allene rearrangement and subsequent Myers–Saito cyclization of β-keto cyclic enediyne.[1]

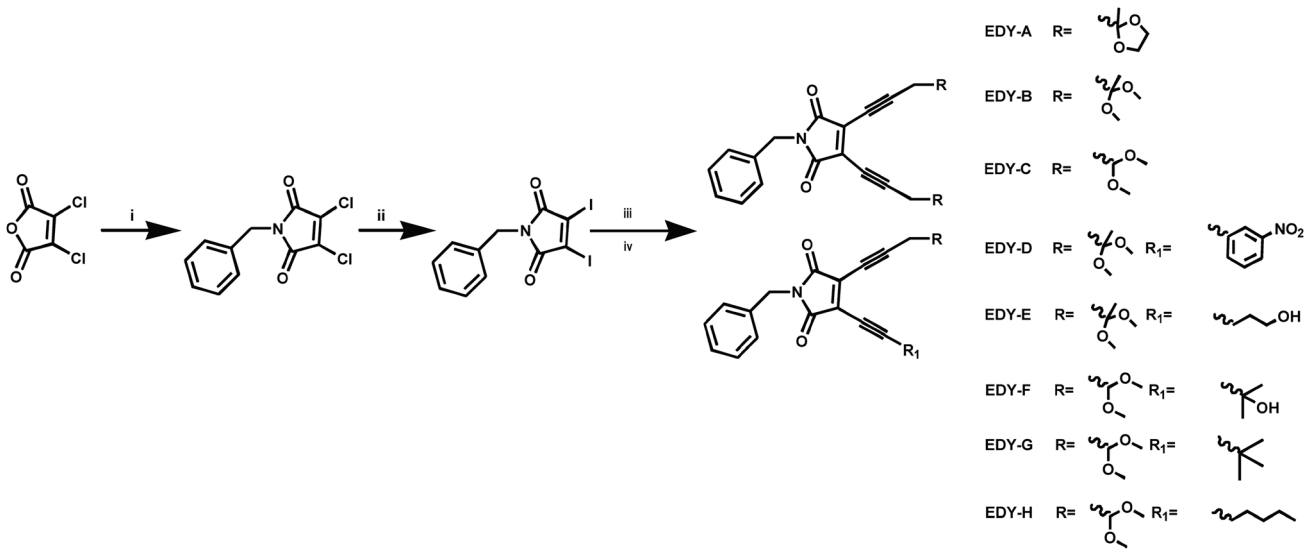
Results and discussion

Design and synthesis of EDY systems with a locking device

Ketal and acetal are commonly applied carbonyl protecting groups for pH-sensitive materials and they show distinct pH-dependent hydrolysis behaviors.[58–61] Meanwhile, the transformation of EDY to reactive enyne-allene requires only one of the alkynes to undergo propargyl-allene tautomerization. Considering these molecular design guidelines, eight EDY compounds (Scheme 2) with either symmetrical (A–C) or asymmetrical (D–H) structures were designed and synthesized. The variable substituents on the asymmetrical EDYs would enable the modulation of the reactivity and the introduction of further functionalities into EDY compounds. Terminal alkynes containing protected carbonyl groups were firstly prepared from the corresponding esters following the method described by Hinkle *et al.*[62] (Scheme S1, ESI†). Sonogashira coupling reactions between diiodomaleic imide[63–65] and slightly excess terminal alkynes were then performed at 40–50 °C to give the symmetrical EDYs in moderate to high yields. For the asymmetric EDYs, an equivalent amount of a second alkyne was added as well. All the obtained EDYs were characterized by ^{1}H NMR, ^{13}C NMR and high resolution mass spectrometry.

Hydrolysis study on a model EDY and identification of enyne-allene products

EDY-A was chosen as a model compound to study the hydrolysis reaction of the protected carbonyl groups. The hydrolysis of **EDY-A** was carried out in the presence of TFA (10 v/v%) in dichloromethane at 0 °C. After complete consumption of **EDY-A**, the reaction mixture was directly subjected to column chromatography to separate out the products. And as shown in Fig. 1, enyne-allene compounds **1** and **2** were obtained (total yield around 10%) and their structures were identified.



Scheme 2 Synthesis of enediyne compounds. (i) Benzylamine, acetic acid, 40 °C, 48 h; (ii) NaI, acetonitrile, 100 °C, 6 h; (iii) synthesis of symmetrical enediyne compounds: alkynes S2, S5, or S7 (3 equiv.), CuI, $Pd(PPh_3)_2Cl_2$, K_2CO_3, tetrahydrofuran, 40–50 °C, overnight; (iv) synthesis of asymmetrical enediyne compounds: alkyne S5 or S7 (1.5 equiv.) with another kind of alkyne (1.5 equiv.), CuI, $Pd(PPh_3)_2Cl_2$, K_2CO_3, tetrahydrofuran, 40–50 °C, overnight.

As mentioned above, after unlocking the β-carbonyl groups in EDY through hydrolysis, a series of reactions continues: the tautomerization of propargyl moieties to produce reactive enyne-allene and the spontaneous Myers–Saito cyclization of enyne-allene to generate diradical intermediates. Additionally, the highly reactive diradical intermediates would react through multiple pathways, such as intramolecular or intermolecular radical transfer and radical coupling reactions to form polymers.[14,26,66] The presence of these reactions in the mixture was believed to contribute to the low yields of **1** and **2** as well as the difficulty in isolating and identifying other small molecular products. Indeed, even compounds **1** and **2** are not very stable. They did not survive during other characterization procedures like HRMS except for NMR spectroscopy.

The ^{1}H NMR spectra of **EDY-A** and compounds **1** and **2** are shown in Fig. 1. Compared to the spectrum of **EDY-A**, the appearance of the characteristic signals at 6.65 (H_{2b}), 6.62 (H_{2c}) and 2.30 ppm (H_{2d}) of the allene ketone structure[67] and the remaining but weakened peaks at 4.00 (H_{2f}), 2.84 (H_{2e}) and 1.47 (H_{2f}) in the spectrum of **1** suggests that the hydrolysis of cyclic ketal and the subsequent rearrangement of propargyl only occur in one of the alkynyl branches of **EDY-A**. As for compound **2**, besides the signals of allene ketone, new peaks corresponding to the propargyl ketone structure emerge, suggesting the hydrolysis of both ketal groups in **EDY-A** with the tautomerization of one of the propargyl ketone moieties. Altogether, this experiment unambiguously confirmed the hydrolysis and tautomerization of these kinds of EDY compounds under acidic conditions to form reactive enyne-allene structures.

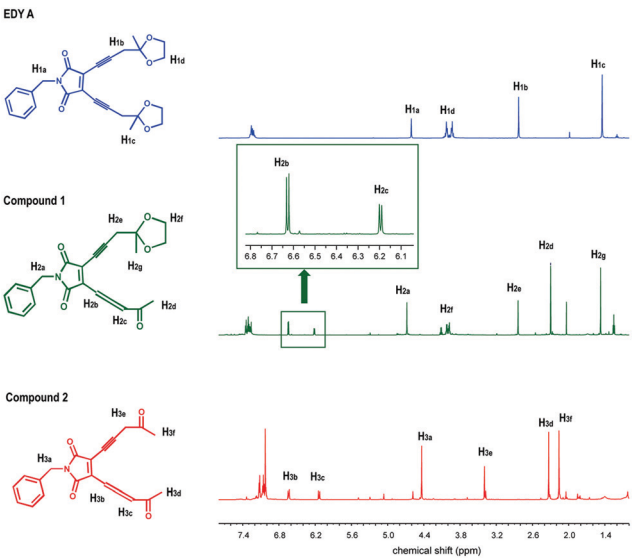
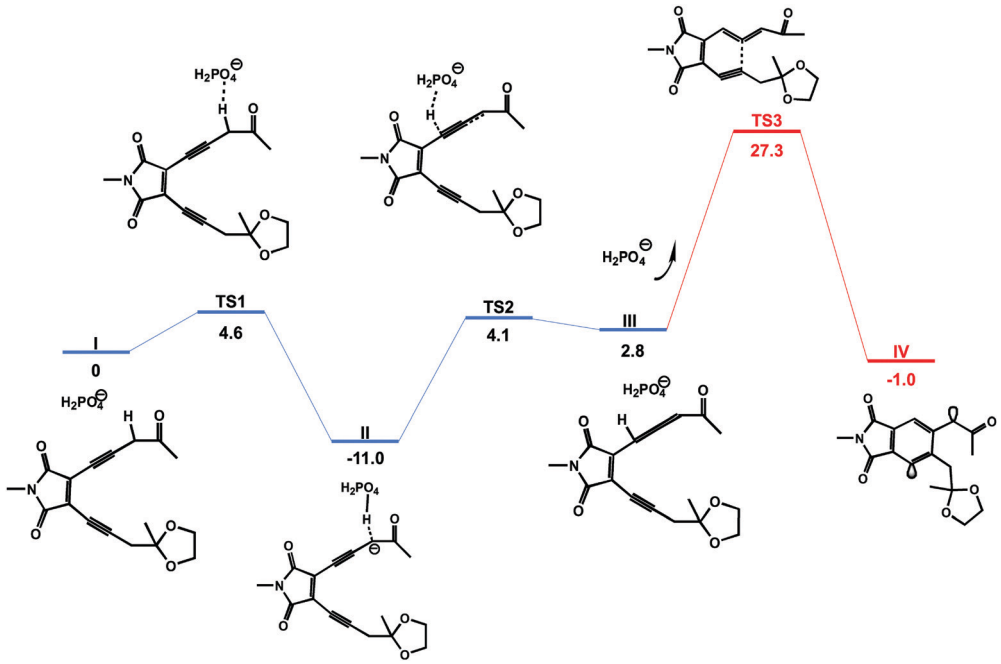


Fig. 1 Comparison of the ^{1}H NMR spectra of **EDY-A** and compounds **1** and **2**.

Computational calculations on a model EDY

To disclose the reaction mechanism for this hydrolysis-induced formation of enyne-allene structures and the subsequent Myers–Saito reaction, density functional theory (DFT) calculations on a partially hydrolyzed structure (similar to compound **1** but with the benzyl group at the maleimide moiety changed to the methyl group to reduce calculation burden) were performed in the GAUSSIAN09 program[68] at the (U)B3LYP/6-31G(d) level with Grimme's dispersion correction.[69] Our previous work has already demonstrated that such a computational setup is suitable for EDY systems.[70] An unrestricted DFT method together with broken symmetry ansatz was only employed in the structure optimizations of the transition state in Myers–Saito cyclization and the resulting diradical product. It turned out that only the diradical has the open shell characteristic. Harmonic vibration frequency calculations were carried out at 298.15 K and the optimized structures are all shown to be either



Scheme 3 Gibbs free energy (kcal mol^{-1}) profile of the reaction pathways at 298.15 K for model compound **I** (blue for propargyl-allene rearrangement in the presence of a dihydrogen phosphate anion; red for Myers–Saito cyclization).

minima (with no imaginary frequency) or transition states (with only 1 imaginary frequency). Further intrinsic reaction coordinate calculations confirmed that all the transition states correspond to the reactions of our interest. A dihydrogen phosphate anion (important and available anion in the biosystem) was applied to serve as the base to assist the tautomerization. As shown in Scheme 3, the tautomerization of **I** to enyne-allene **III** occurs through 1,3-proton transfer assisted by a dihydrogen phosphate anion. Over transition state **TS1**, the propargyl proton of **I** is abstracted by the anion, leading to intermediate **II** with a propargyl carbanion. Subsequently, over the transition state **TS2** yields the enyne-allene **III**. It is worth mentioning that Gibbs free energy barriers for this transformation assisted by a dihydrogen phosphate anion are quite low (4.6 kcal mol^{-1} and 15.1 kcal mol^{-1} respectively). After the formation of enyne-allene, further calculation indicates that the diradical was generated by Myers–Saito cyclization *via* **TS3** (24.5 kcal mol^{-1} energy barrier for **III** to form **IV**). This result further suggests that the designed EDY system is capable of transforming into reactive enyne-allene and undergoing Myers–Saito cyclization under physiological conditions.

EPR study on acid-triggered radical generation

After removal of the locking substituents on the carbonyl groups and the generation of enyne-allene structures, all eight EDYs are ready to undergo Myers–Saito cyclization at ambient temperature to produce reactive diradicals. The reactive diradical species are generally trapped by spin trapping agents to generate stable free radicals, which could be analyzed with electron paramagnetic resonance (EPR) spectroscopy.[64,71] Alternatively, when trapped by some stable radicals, like tetramethylpiperidine oxide (TMEPO), a decrease of radical signals in EPR spectroscopy is displayed. To study the generation of reactive free diradicals from these EDYs, their solutions in DMSO were mixed with PBS solutions of pH 5.5 or pH 7.5 in the presence of TEMPO respectively. Samples without additional EDY were used as the control. EPR spectra were recorded right after the mixing of these species (0 h) and after 24 h of incubation. As shown in Fig. 2, more significant reductions in nitroxide radical signals are observed for all EDY systems at pH 5.5 at 24 h in comparison with EDY systems at pH 7.5. The most remarkable reduction of radical signals of TEMPO was observed when **EDY-G** was added under acidic conditions. As shown in Fig. 2, it is clear that the rate of free radical generation is also affected by the substitutes on the non-sensitive branch in asymmetric EDY.

Evaluation of the DNA-cleaving ability of EDYs

The acid-triggered radical generation of these EDYs endows them with DNA-scissoring ability. To evaluate their DNA cleavage performance, EDYs were incubated with supercoiled plasmid form **I** PUC19 DNA under different conditions and then subjected to gel electrophoresis. The amount of the scissored DNA in circular relaxed form **II** was determined with densitometric analysis of the gel electrophoresis bands. As shown in Fig. 3, EDYs are capable of cleaving DNA in a concentration and pH dependent manner (Fig. 3I and II). At pH 5.5, all EDYs exhibit enhanced DNA-cleaving ability compared with samples at pH 7.5, however the enhancements for EDYs were quite different. For example, only a 17% increase in DNA cleavage was observed for **EDY-A** at 2 mM, while for **EDY-B** the increase was up to 900% (Table S1, ESI†). This phenomenon was attributed to the distinct hydrolysis behavior of carbonyl-protective groups that affects the tautomerization of the propargyl group and the

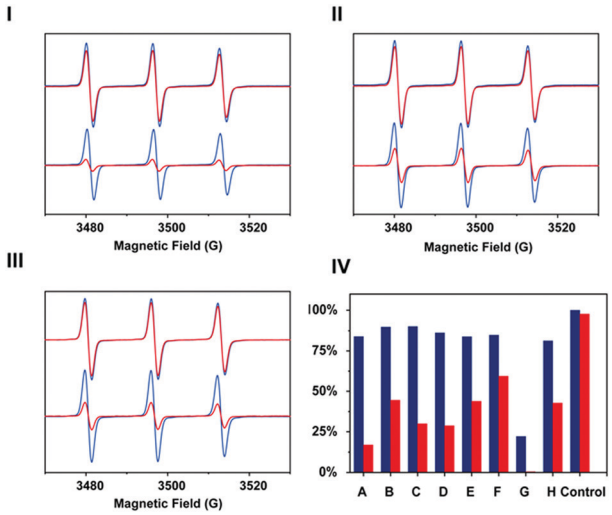


Fig. 2 (I)–(III) EPR spectra of **EDY-A**, **EDY B** and **EDY C** in the presence of TEMPO at 0 h (blue lines) and 24 h (red lines) at pH 7.5 (upper lines) and 5.5 (lower lines); and (IV) relative intensities of TEMPO signals at 24 h at pH 7.5 (blue) or pH 5.5 (red) for EDYs and the control compared to those at 0 h.

subsequent Myers–Saito cyclization. It is well documented that acyclic ketal groups are more prone to hydrolysis in comparison with cyclic ketal groups.[57,72,73] Further DNA-cleaving experiments were performed on **EDY-A**, **C**, **E** and **F** at a concentration of 2 mM at different pH values from 5.5 to 8 (Fig. 3III and IV). A general trend is observed that EDYs with acetal protecting groups are more sensitive to acidic environments than those with ketal protecting groups. The reason for the relatively high DNA-cleaving ability of **EDY-A** under neutral to basic conditions is still unknown, which might relate to the Bergman or Bergman-like reaction similar to the EDYs in our previous work.[63–65]

Among all the eight EDYs, **EDY-G** exhibited the best performance in DNA cleaving experiments (Fig. 3II), which was consistent with its highest free radical generating rate as shown in the EPR measurements above. We found that even when diluted to 0.25 mM, **EDY-G** produced a significant amount of DNA cleavages at pH 5.5 (Fig. 4I and II). Further DNA-cleaving experiments were performed on **EDY-G** to identify the reactive species involved. To this end, pyridine, methanol or DL-dithiothreitol (DTT) was used to track the involvement of alkylating species, hydroxyl radicals or singlet oxygen[74] accordingly. As shown in Fig. 4III, no DNA-protecting effect was demonstrated by pyridine or methanol, while in the presence of DTT, less amount of form **II** DNA was observed, suggesting that singlet oxygen was involved in the pH-dependent DNA-cleaving process of EDYs. The singlet oxygen is believed to result from the radical transfer between the EDY-produced diradical intermediates and oxygen molecules.

MTT assay

The anti-proliferation ability of the EDYs against HeLa cells was evaluated with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay. As shown in Fig. 5, all the EDYs demonstrate cytotoxicity in a concentration-dependent manner after 48 h incubation with HeLa cells. Furthermore, most of the EDYs exhibit significant anti-proliferation ability against HeLa cells (Table S2, ESI†). The relatively high IC_{50} of **EDY-A** (22.20 μM) is probably due to the rather slow hydrolysis process[57] of cyclic-ketal protecting groups. Overall, **EDY-B** showed the highest cytotoxicity towards HeLa cell with an IC_{50} value of 1.40 μM which is comparable to those of most of the clinically applied antitumor agents such as doxorubicin and cisplatin.[64]

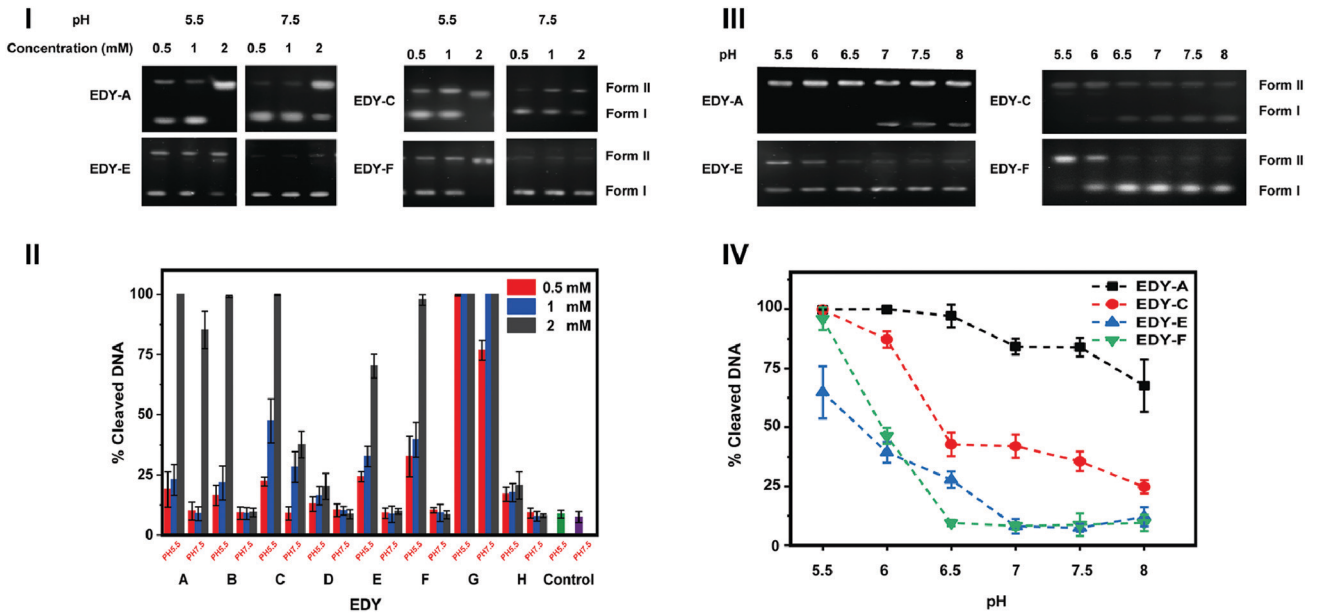


Fig. 3 (I) DNA cleavages of **EDY-A**, **C**, **E** and **F** at different concentrations and pH; (II) quantified DNA cleavage percentages for EDYs and the control (red for 0.5 mM, blue for 1 mM and grey for 2 mM) at pH 5.5 or 7.5. Reported values represent at least three experiments; and (III) and (IV) DNA cleavages and quantified DNA cleavages of **EDY-A**, **C**, **E** and **F** at pH 5.5 to 8.0.

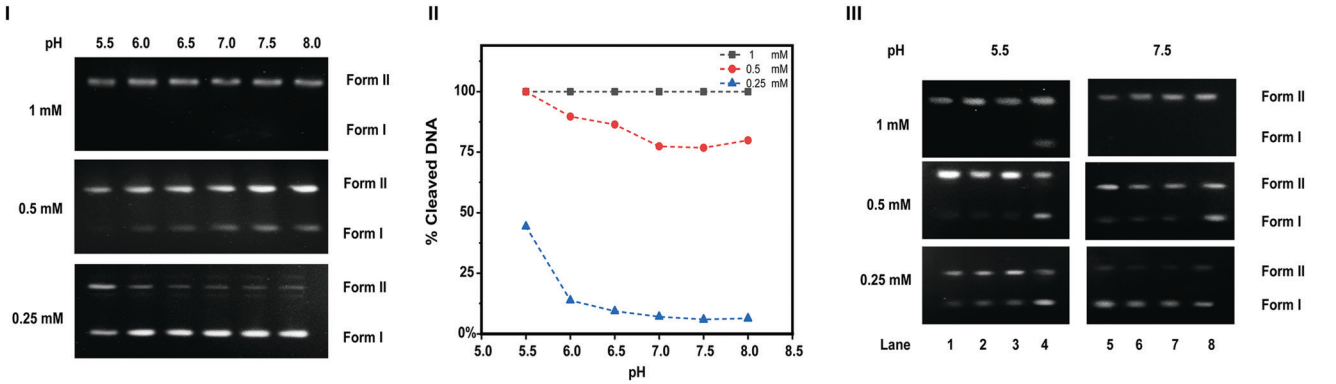


Fig. 4 (I and II) DNA cleavages and quantified DNA cleavages by 1, 0.5 and 0.25 mM **EDY-G** at pH 5.5 to 8.0; and (III) DNA cleavage by 1, 0.5 and 0.25 mM **EDY-G** in the presence of different agents. Lanes 1 and 5: blank; lanes 2 and 6: pyridine; lanes 3 and 7: methanol; and lanes 4 and 8: DTT.

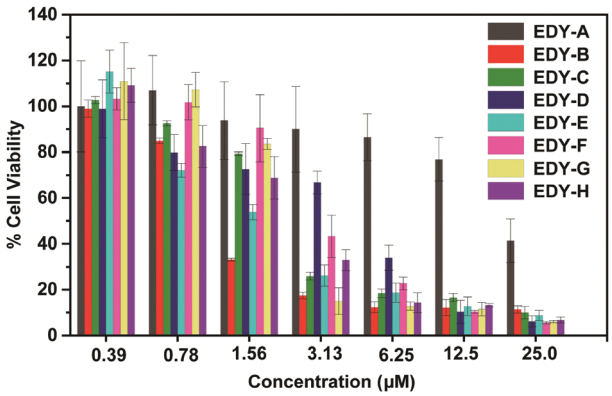


Fig. 5 *In vitro* cytotoxicities of EDYs against HeLa cells determined by the MTT assay. The data are presented as the average value of at least three experiments.

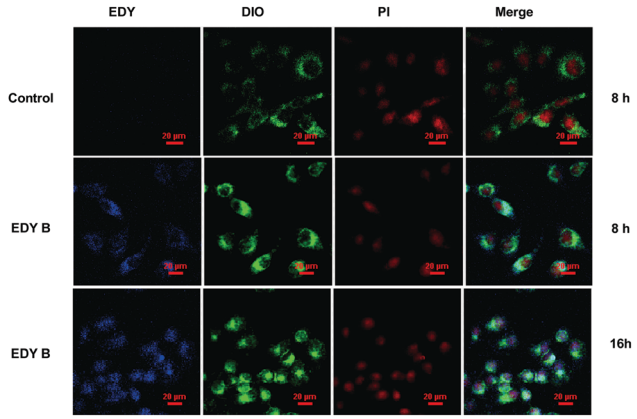


Fig. 6 Fluorescence confocal microscopy images of HeLa cells treated with **EDY B** for 8 h or 16 h. The control is HeLa cells incubated without any treatment. DIO (green) and PI (red) are used as indicators for the cell membrane and nucleus respectively.

In vitro internalization and localization of EDY

To investigate the cytotoxicity mechanism of EDYs, the cellular internalization and distribution processes of EDY were studied with fluorescence confocal microscopy. The inherent blue fluorescence emission (Fig. S1, ESI†) of **EDY-B** enables the direct tracking of it through confocal microscopy. In these experiments, HeLa cells were treated with 2 μM EDY-B for 8 h or 16 h and were stained with 3,3′-dioctadecyloxacarbocyanine perchlorate (DIO, green) and propidium iodide (PI, red) to indicate the cell membrane and nucleus, respectively. As shown in Fig. 6, efficient internalization of **EDY-B** is achieved after 8 h incubation, however **EDY-B** mainly appears in the cytoplasm, where only slight overlap of blue fluorescence from EDY and red fluorescence from PI (nucleus) is observed. With increasing incubation time of 16 h, **EDY-B** is found to accumulate in the cell nucleus, suggesting the contact between EDY and DNA which facilitates the DNA-cleavage process as discussed below.

In vitro DNA damage and ROS detection

As a class of radiomimetic chemotherapeutic agents, the observed cytotoxicity of EDY compounds is generally considered to originate from their DNA damaging ability. The DNA damage caused by **EDY-B** was evaluated intracellularly by fluorescence confocal microscopy using a DNA damage biomarker gamma-H2AX. Fig. 7I shows that DNA damages are generated when HeLa cells are treated with **EDY-B**. Moreover, the intracellular DNA damage shows a dose-dependent trend, similar to its extracellular DNA-cleaving behavior discussed above. The above-mentioned extracellular experiments showed that a radical mechanism including singlet oxygen was involved in the EDY-induced DNA cleavage. Consequently, the intracellular DNA damaging ability of EDY shall relate to the singlet oxygen production, which suggests that an elevated ROS (reactive oxygen species) level should be observed *in vitro*. To validate this, HeLa cells incubated with 5 μM **EDY-B** were subjected to fluorescence confocal microscopy using a ROS indicator, 2,7-dichlorodihydrofluorescein (DCF). As demonstrated in Fig. 7II, the green fluorescence increases dramatically in the presence of EDY when compared with the control sample. Meanwhile, the distribution of green fluorescence significantly overlaps with the blue fluorescence from EDY, indicating that the designed EDYs are able to enhance the ROS level inside cells, resulting in DNA damage and cell death.

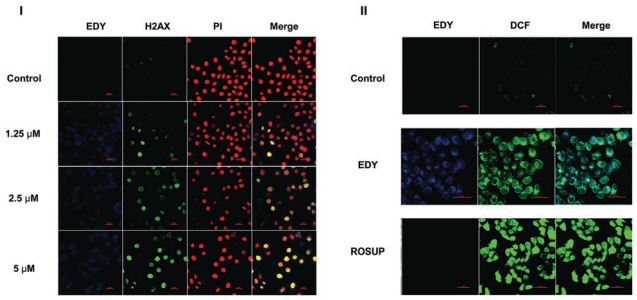


Fig. 7 (I) *In vitro* DNA damage (H2AX as an indicator, green) induced by **EDY-B** (0, 1.25, 2.5, and 5 μM) and; (II) *in vitro* ROS level (DCF as an indicator, green) induced by **EDY-B** (0, 5 μM). HeLa cells were treated with ROSUP as the positive control.

Cell cycle arrest and apoptosis induced by EDY

DNA damage may activate multiple checkpoints that delay cell-cycle progression and trigger DNA repair, resulting in cell cycle arrest.[75] As most DNA-targeted chemotherapeutic drugs demonstrate their cell cycle arresting ability, we explored whether the designed EDYs are able to interfere in the cell cycle progression by flow cytometry. The results are shown in Fig. 8I and summarized in Fig. S2 (ESI†). With the addition of EDY, S phase accumulation is realized, showing the percentage of S phase increases from 18.6% to 27.7% with the addition of 1.25 μm **EDY-B**. Furthermore, the S phase accumulation of HeLa cells increases slightly with concentration increase of **EDY-B**, accompanied by the decrease of the G2/M phase. Altogether, **EDY-B** demonstrates an S phase arresting behavior, which has been observed in a natural EDY chemotherapeutic agent NCS as well.[76]

Under severe DNA damage, programmed apoptosis can be activated besides the disordered cell cycle progression.[76] Annexin V/PI staining assay was conducted to investigate the EDY-induced cell death pathway. With increased concentration of **EDY-B**, significantly improved apoptosis rates are observed compared with the control, and a high apoptosis rate of about 95% is achieved with the addition of 5 μM **EDY-B** (Fig. 8II and Fig. S3, ESI†). This significantly high apoptosis rate induced by the newly designed EDYs further corroborates their high efficiency, signaling great potential in chemotherapy and cancer curing.

Conclusions

We have synthesized a series of EDYs with different locking groups (ketal and acetal), which would hydrolyze under acidic conditions to form enyne-allene structures and further undergo Myers–Saito cyclization to produce reactive diradical intermediates. The generation of radical species was confirmed with EPR analysis and their DNA-cleaving ability was evaluated with gel electrophoresis, both of which exhibited dose and pH dependent behaviors. We also demonstrated that the pH-response window and the reactivity of EDYs can be modulated with the variation of carbonyl-locking groups. Most EDYs possess promising cytotoxicity with IC_{50} values below 5 μM, comparable to those of many clinically used antitumor agents. A further study on HeLa cells revealed that the designed EDYs induced DNA damage intracellularly through a radical-involved mechanism, which caused cell cycle arrest and cell death through the apoptosis mechanism. Overall, the design of pH-sensitive EDY systems that take advantage of the relatively acidic microenvironment of tumor cells to ignite the Myers–Saito cyclization would shed new light on the development of NCS-mimic chemotherapeutic agents for clinical applications.

Conflicts of interest

There are no conflicts to declare.

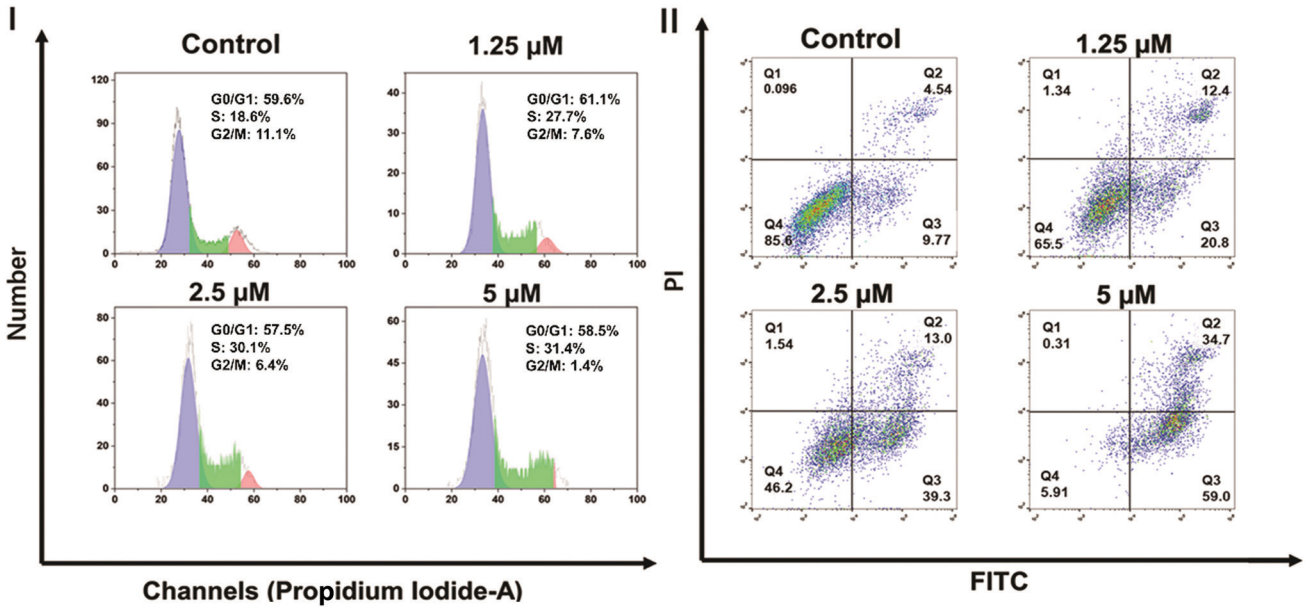


Fig. 8 Cell cycle distributions and apoptosis of HeLa cells treated with **EDY-B** of indicated concentrations.

Acknowledgements

The authors gratefully acknowledge the financial support from the National Natural Science Foundation of China (21871080, 21503078, and 21474027), the Fundamental Research Funds for the Central Universities (22221818014), and the Shanghai Leading Academic Discipline Project (B502). AH thanks the "Eastern Scholar Professorship" support from Shanghai local government.

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