Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/d1ob01089a

Received 5th June 2021,
Accepted 1st July 2021

DOI: 10.1039/d1ob01089a

rsc.li/obc

A triple-targeting delivery system carrying two anticancer agents†

Hui Li, Chang-Hee Lee and Injae Shin *

To improve tumor selectivity, a triple-targeting delivery system (Oct-FK(PBA-Az)-Dox) carrying two anticancer agents (apoptozole (Az) and doxorubicin (Dox)) was designed and synthesized. The results showed that both anticancer agents in Oct-FK(PBA-Az)-Dox are liberated in the presence of both H_2O_2 and cathepsin B, which are normally present at high levels in tumors.

Over the past several decades, a variety of potential chemotherapeutic agents have been developed to treat tumors.[1] A major goal of these efforts is the discovery of safe and efficacious anticancer drugs, which have enhanced selectivities against cancer cells over normal cells and, thus, display minimal side effects.[2] In an effort to improve the tumor selectivity of anticancer agents, drug delivery systems that target cell-surface receptors and/or enzymes overexpressed in cancer cells have been developed.[3] This approach contributes to improving the selectivity of cancer chemotherapeutic agents. However, the tumor selectivities of drug delivery systems developed to date are still not high and their cross-reactivity with normal cells remains problematic. To overcome the limitations of current drug delivery systems and further improve tumor selectivity, we designed and synthesized the new triple-targeting delivery system Oct-FK(PBA-Az)-Dox, which contains two anticancer agents. The results of this investigation showed that both anticancer agents in this substance are released in the presence of both H_2O_2 and cathepsin B, each of which is produced at high levels in cancer cells.

Somatostatin receptors (SSTRs) are upregulated in many types of tumors.[4] Particularly, SSTR2 is most frequently overexpressed on the surfaces of various cancer cells and, thus, is a good target for agents that selectively deliver drugs to tumors. Octreotide (Oct), a synthetic cyclic octapeptide which pharmacologically mimics the natural ligand somatostatin,[5] has been utilized as a ligand for targeting SSTRs in delivery systems. This synthetic peptide recognizes SSTR2 relatively selectively over other SSTRs and its conjugates with anticancer or imaging agents enter cells *via* SSTR-mediated endocytosis.[6] Thus, SSTRs were chosen as the 1st target of the delivery system developed in this study (Fig. 1).

Several previous studies have shown that H_2O_2 is present at high levels in most types of tumors.[7] On this basis, H_2O_2 was chosen as the 2nd target of the new targeting delivery system. Furthermore, the cysteine protease cathepsin B, present in lysosomes, is upregulated in various tumors, and is involved in tumor invasion and metastasis.[8] This protease is also often upregulated in premalignant lesions and is involved in local invasive stages of tumors.[8] Accordingly, the lysosomal cathepsin B was selected as the 3rd target of our new delivery system.

In previous studies, we showed that apoptozole (Az), an inhibitor of Hsp70, induces lysosomal membrane permeabilization and thereby enhances lysosome-mediated apoptotic cancer cell death (Fig. 2a).[9] In addition, doxorubicin (Dox, an inhibitor of topoisomerase II) displays potent anticancer activity against various cancer cells.[10] Furthermore, we also showed that the combined treatment of Az and Dox promotes enhanced apoptosis of cancer cells.[9a] As a result, Az and Dox

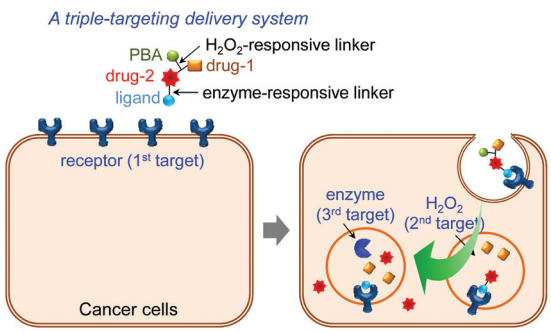


Fig. 1 Schematic representation of the cancer cell-specific triple-targeting delivery system carrying two anticancer agents.

Department of Chemistry, Yonsei University, Seoul 03722, Republic of Korea.
E-mail: injae@yonsei.ac.kr

†Electronic supplementary information (ESI) available. See DOI: 10.1039/d1ob01089a

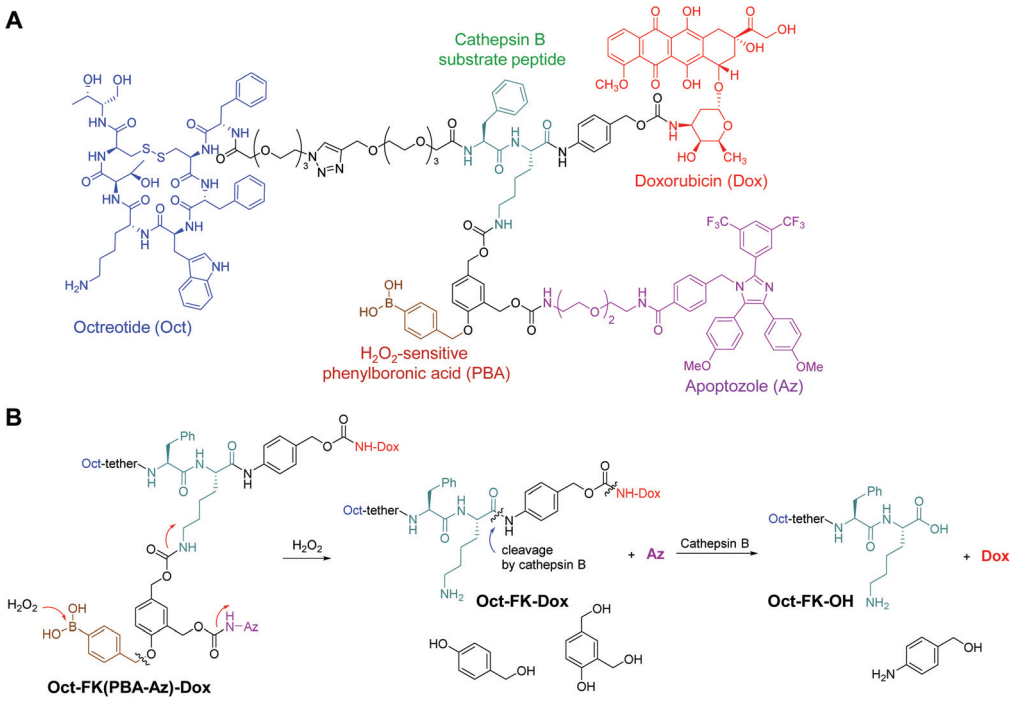


Fig. 2 (A) Structure of the triple-targeting delivery system (Oct-FK(PBA-Az)-Dox). (B) Sequential cleavage of Oct-FK(PBA-Az)-Dox by H_2O_2 and cathepsin B to liberate Az and Dox.

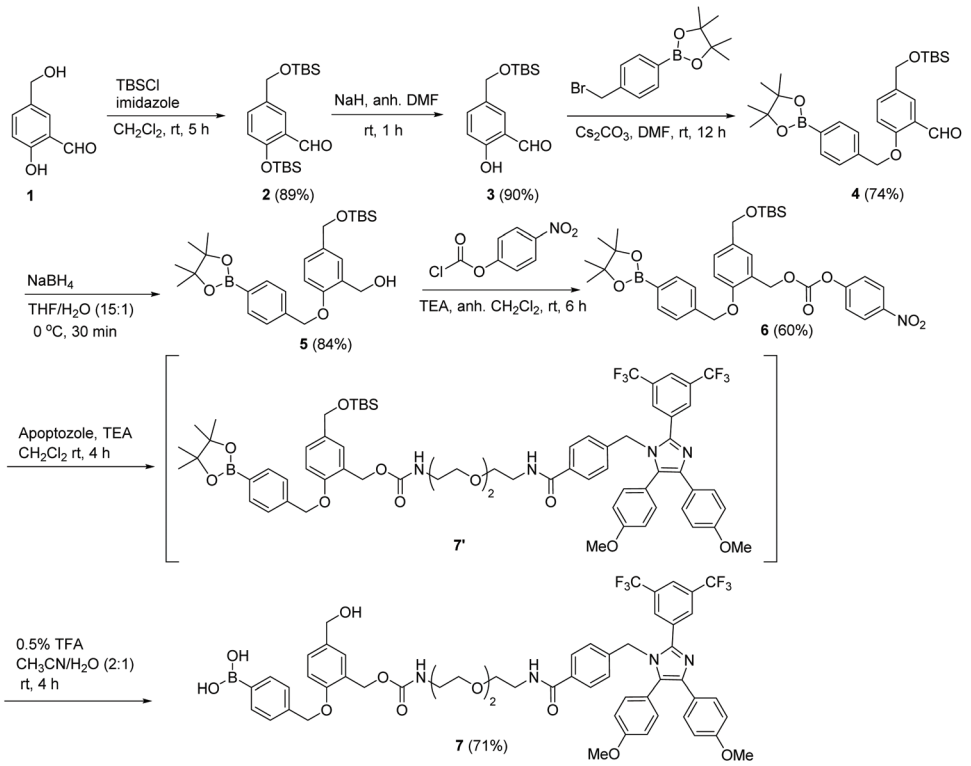
were utilized as anticancer agents in the new triple-targeting delivery system.

Utilizing the strategy and components described above, we designed the triple-targeting delivery system Oct-FK(PBA-Az)-Dox (Fig. 2a). The system consists of (1) Oct acting as an SSTR ligand, (2) a H_2O_2-responsive phenylboronic acid (PBA) moiety, (3) a dipeptide Phe-Lys (FK) serving as a cathepsin B substrate, and (4) two anticancer agents. Because they are covalently linked to the delivery system, both Dox and Az in Oct-FK(PBA-Az)-Dox are expected to have almost no antitumor activity. It is anticipated that following its binding through Oct to upregulated SSTRs, Oct-FK(PBA-Az)-Dox will be internalized into lysosomes of cancer cells *via* SSTR-mediated endocytosis. The PBA moiety of Oct-FK(PBA-Az)-Dox will then be cleaved by reaction with H_2O_2 present at high levels in cancer cells, concomitantly releasing the anticancer agent Az and producing Oct-FK-Dox (Fig. 2b). Subsequently, the cathepsin B induced cleavage of the C-terminus of the dipeptide in Oct-FK-Dox will lead to the release of Dox. Free Az and Dox will kill cancer cells effectively and selectively.

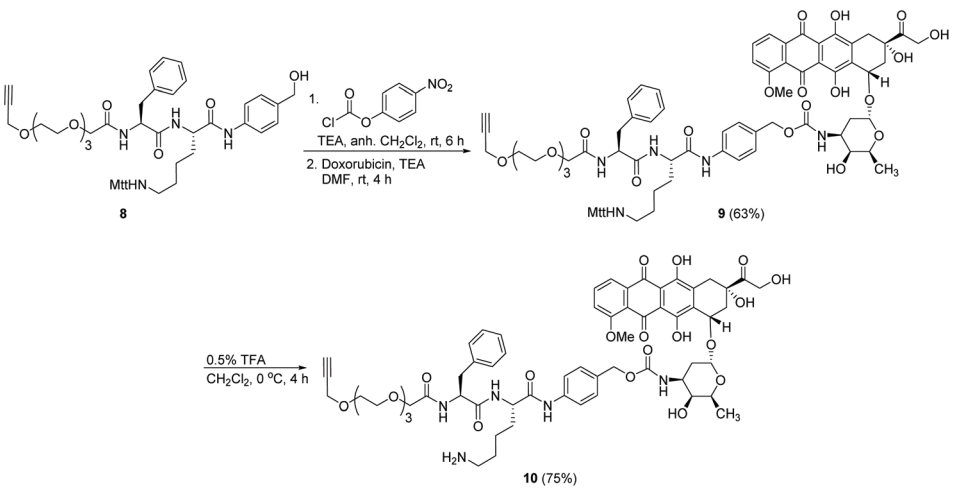
To assess this proposal, the triple-targeting delivery system, Oct-FK(PBA-Az)-Dox, was prepared by using the route shown in Schemes 1–3. In the sequence, the two hydroxyl groups in the benzaldehyde derivative **1** were first protected with TBS to generate **2** (Scheme 1). The phenolic TBS group in **2** was selectively removed by reaction with NaH to form a mono-TBS protected product **3**.[11] The reaction of **3** with 4-bromomethylphenylboronic acid pinacol ester under basic conditions generated boronate **4**. Reduction of **4** with $NaBH_4$ produced **5**, which reacted with *p*-nitrophenyl chloroformate to afford a mixed carbonate **6**. To prepare the Az-conjugated compound **7′**, **6** was reacted with Az and the crude mixture was subjected to flash column chromatography. It was found that the phenylboronate ester moiety in **7′** is partially hydrolyzed to form phenylboronic acid (PBA) during purification by flash column chromatography. Thus, both TBS and pinacol ester groups in **7′** were removed under weakly acidic conditions to afford **7** that was readily purified by flash column chromatography.

The Dox-conjugated intermediate **10** was synthesized from **8** which was prepared by using a known procedure (Scheme 2).[6a] Specifically, the benzyl alcohol moiety in **8** reacted with *p*-nitrophenyl chloroformate to form a mixed carbonate, which upon treatment with doxorubicin produced **9**. The 4-methoxytrityl (MTT) protecting group in **9** was carefully removed under weakly acidic conditions to yield intermediate **10**. It should be noted that the glycosidic linkage in Dox is cleaved when more strongly acidic conditions are employed.

Coupling of **7** to **10** was then accomplished by initially activating the hydroxyl group in **7** with *p*-nitrophenyl chloroformate and subsequent reaction of the resulting carbonate with **10** to produce **11** (Scheme 3). Finally, the alkynylated compound **11** was subjected to click chemistry with azide-appended Oct (Oct-N_3), which was synthesized by using conventional Fmoc/*t*Bu solid-phase peptide synthesis (Scheme S1†),[6a] to form Oct-FK(PBA-Az)-Dox. The reaction mixture containing Oct-FK(PBA-Az)-Dox was purified by preparative HPLC and analysed by MS. Also, all intermediates in the synthetic pathway were characterized by NMR and MS.



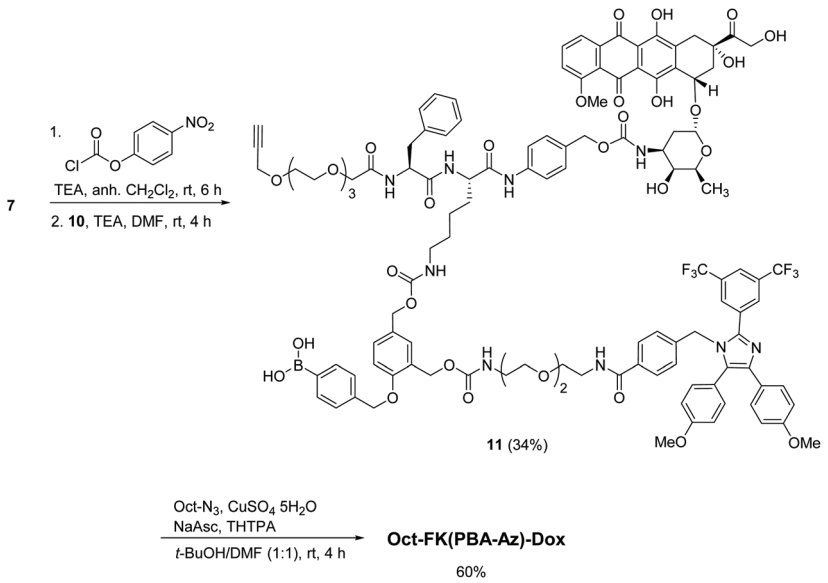
Scheme 1 Synthesis of compound **7**.



Scheme 2 Synthesis of compound **10**.

We then explored the release of Dox and Az from Oct-FK(PBA-Az)-Dox by reversed-phase HPLC analysis of mixtures of Oct-FK(PBA-Az)-Dox treated with H_2O_2 and/or cathepsin B (Fig. 3). The findings showed that the incubation of Oct-FK(PBA-Az)-Dox with H_2O_2 leads to the production of Az and Oct-FK-Dox (Fig. 2B). However, neither Az nor Dox was released when a mixture of Oct-FK(PBA-Az)-Dox was treated with cathepsin B, indicating that this substance possessing a PBA-capped lysine is not hydrolytically cleaved by the enzyme. Importantly, both Dox and Az, along with Oct-FK-OH, were liberated from Oct-FK(PBA-Az)-Dox by sequential treatment with H_2O_2 and cathepsin B. In contrast, free Dox was not generated when Oct-FK(PBA-Az)-Dox was sequentially incubated with H_2O_2 and cathepsin B in the presence of CA-074-Me, a selective inhibitor of cathepsin B.[12] The findings clearly indicate that both Dox and Az are released from the new delivery system only in the presence of H_2O_2 and cathepsin B.

In conclusion, we designed and prepared a novel delivery system containing a triple-targeting site composed of a tumor-



Scheme 3 Synthesis of Oct-FK(PBA-Az)-Dox.

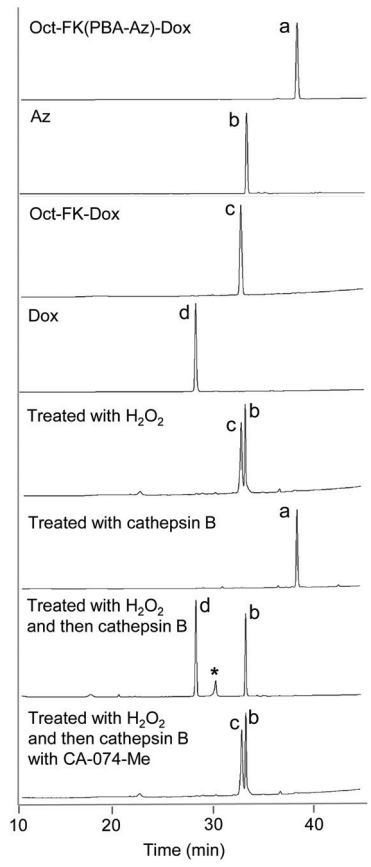


Fig. 3 Release of Az and Dox from Oct-FK(PBA-Az)-Dox by treatment with H_2O_2 or/and cathepsin B. Oct-FK(PBA-Az)-Dox was treated with H_2O_2 or cathepsin B alone. In addition, Oct-FK(PBA-Az)-Dox was treated with H_2O_2 followed by cathepsin B in the absence or presence of the cathepsin B inhibitor CA-074-Me. Reaction mixtures were analyzed by reverse-phase HPLC (detection at 250 nm). Oct-FK(PBA-Az)-Dox (a), Az (b), Oct-FK-Dox (c) and Dox (d) utilized as controls. Asterisk indicates Oct-FK-OH ([M + Na] m/z = 1777.7).

selective SSTR ligand, a peptide substrate for cathepsin B and a H_2O_2-responsive PBA moiety, along with two anticancer agents (Az and Dox). We demonstrated that both of the linked anticancer agents are released from Oct-FK(PBA-Az)-Dox only in the presence of H_2O_2 and cathepsin B. Cell and *in vivo* studies are now underway to assess the usefulness of a novel delivery system for the treatment of cancer.

Conflicts of interest

The authors declare no competing interests.

Acknowledgements

This study was supported financially by the National Research Foundation of Korea (grant no. 2020R1A2C3003462).

Notes and references

1 (*a*) G. Schneider, *Nat. Rev. Drug Discovery*, 2018, **17**, 97–113; (*b*) M. Dobbelstein and U. Moll, *Nat. Rev. Drug Discovery*, 2014, **13**, 179–196; (*c*) F. M. Ferguson and N. S. Gray, *Nat. Rev. Drug Discovery*, 2018, **17**, 353–377; (*d*) R. L. Schilsky, *Nat. Rev. Drug Discovery*, 2010, **9**, 363–366; (*e*) B. Hughes, *Nat. Rev. Drug Discovery*, 2010, **9**, 665–667; (*f*) K.-H. Baek, J. Park and I. Shin, *Chem. Soc. Rev.*, 2012, **41**, 3245–3263.
2 A. Kamb, S. Wee and C. Lengauer, *Nat. Rev. Drug Discovery*, 2007, **6**, 115–120.
3 (*a*) M. H. Baig, M. Adil, R. Khan, S. Dhadi, K. Ahmad, G. Rabbani, T. Bashir, M. A. Imran, F. M. Husain, E. J. Lee, M. A. Kamal and I. Choi, *Semin. Cancer Biol.*, 2019, **56**, 1–11; (*b*) H.-W. Liu, L. Chen, C. Xu, Z. Li, H. Zhang,

X.-B. Zhang and W. Tan, *Chem. Soc. Rev.*, 2018, **47**, 7140–7180; (*c*) Y. Singh, M. Palombo and P. J. Sinko, *Curr. Med. Chem.*, 2008, **15**, 1802–1826; (*d*) R. V. J. Chari, *Adv. Drug Delivery Rev.*, 1998, **31**, 89–104; (*e*) T. M. Allen, *Nat. Rev. Cancer*, 2002, **2**, 750–763; (*f*) J. Rautio, H. Kumpulainen, T. Heimbach, R. Oliyai, D. Oh, T. Järvinen and J. Savolainen, *Nat. Rev. Drug Discovery*, 2008, **7**, 255–270; (*g*) W. Arap, R. Pasqualini and E. Ruoslahti, *Science*, 1998, **279**, 377–380.

4 (*a*) L.-C. Sun and D. H. Coy, *Curr. Drug Delivery*, 2011, **8**, 2–10; (*b*) M. De Jong, W. A. Breeman, D. J. Kwekkeboom, R. Valkema and E. P. Krenning, *Acc. Chem. Res.*, 2009, **42**, 873–880.

5 M. Gomes-Porras, J. Cárdenas-Salas and C. Álvarez-Escolá, *Int. J. Mol. Sci.*, 2020, **21**, 1682.

6 (*a*) X. Tian, K.-H. Baek and I. Shin, *Chem. Sci.*, 2013, **4**, 947–956; (*b*) H. Li, C.-H. Lee and I. Shin, *Org. Lett.*, 2019, **21**, 4628–4631; (*c*) G. Ösapay, L. Prokai, H.-S. Kim, K. F. Medzihradszky, D. H. Coy, G. Liapakis, T. Reisine, G. Melacini, Q. Zhu, S. H. H. Wang, R.-H. Mattern and M. Goodman, *J. Med. Chem.*, 1997, **40**, 2241–2251; (*d*) F. Barragán, D. Carrion-Salip, I. Gómez-Pinto, A. González-Cantó, P. J. Sadler, R. de Llorens, V. Moreno, C. González, A. Massaguer and V. Marchán, *Bioconjugate Chem.*, 2012, **23**, 1838–1855.

7 (*a*) D. Trachootham, J. Alexandre and P. Huang, *Nat. Rev. Drug Discovery*, 2009, **8**, 579–591; (*b*) G.-Y. Liou and P. Storz, *Free Radical Res.*, 2010, **44**, 479–496; (*c*) T. P. Szatrowski and C. F. Nathan, *Cancer Res.*, 1991, **51**, 794; (*d*) M. López-Lázaro, *Cancer Lett.*, 2007, **252**, 1–8.

8 (*a*) F. Lecaille, J. Kaleta and D. Brömme, *Chem. Rev.*, 2002, **102**, 4459–4488; (*b*) M. M. Mohamed and B. F. Sloane, *Nat. Rev. Cancer*, 2006, **6**, 764–775; (*c*) O. Vasiljeva, T. Reinheckel, C. Peters, D. Turk, V. Turk and B. Turk, *Curr. Pharm. Des.*, 2007, **13**, 387–403; (*d*) J. A. Joyce, A. Baruch, K. Chehade, N. Meyer-Morse, E. Giraudo, F.-Y. Tsai, D. C. Greenbaum, J. H. Hager, M. Bogyo and D. Hanahan, *Cancer Cell*, 2004, **5**, 443–453; (*e*) S. Yan, M. Sameni and B. F. Sloane, *Biol. Chem.*, 1998, **379**, 113–123.

9 (*a*) S.-K. Ko, J. Kim, D. C. Na, S. Park, S.-H. Park, Ji Y. Hyun, K.-H. Baek, N. D. Kim, N.-K. Kim, Y. N. Park, K. Song and I. Shin, *Chem. Biol.*, 2015, **22**, 391–403; (*b*) D. R. Williams, S.-K. Ko, S. Park, M.-R. Lee and I. Shin, *Angew. Chem., Int. Ed.*, 2008, **47**, 7466–7469; (*c*) S.-H. Park, K.-H. Baek, I. Shin and I. Shin, *Cell Chem. Biol.*, 2018, **25**, 1242–1254.

10 (*a*) K. Tewey, T. Rowe, L. Yang, B. Halligan and L. Liu, *Science*, 1984, **226**, 466–468; (*b*) J. C. Wang, *Annu. Rev. Biochem.*, 1996, **65**, 635–692; (*c*) J. L. Nitiss, *Nat. Rev. Cancer*, 2009, **9**, 338–350.

11 R. A. Fernandes, S. P. Gholap and S. V. Mulay, *RSC Adv.*, 2014, **4**, 16438–16443.

12 T. Towatari, T. Nikawa, M. Murata, C. Yokoo, M. Tamai, K. Hanada and N. Katunuma, *FEBS Lett.*, 1991, **280**, 311–315.