NQO1-Selective Activated Prodrug of Triptolide: Synthesis and Antihepatocellular Carcinoma Activity Evaluation

Meilin Liu,[†] Wei Song,[†] Xi Du, Jingtian Su, Kang Dong, Yong Chen,* and Zhihong Peng*^{ID}

School of Life Sciences, Hubei Province Key Laboratory of Biotechnology of Chinese Traditional Medicine, National & Local Joint Engineering Research Center of High-throughput Drug Screening Technology, Hubei University, Wuhan 430062, China

Supporting Information

ABSTRACT: Hepatocellular carcinoma (HCC) is the leading cause of death in patients with cirrhosis. Due to its poor response to conventional chemotherapy drugs, the prognosis for its survival is the worst. NAD(P)-H:quinone oxidoreductase 1 (NQO1) is an attractive anticancer target due to its overexpression in HCC. Although triptolide (TP) possesses potent antitumor activity, its clinical practice is greatly limited due to its general toxicities and narrow therapeutic window. Herein, we develop an NQO1-selective activated TP analog, named CX-23, which exhibited antiproliferation of HepG2 over normal hepatocytes in vitro. In vivo study shows that CX-23 can not only prevent the hepatocellular carcinoma progression but also migrate the liver and kidney toxicity. These findings indicate that NQO1 may serve as a targeted delivery system to release an antitumor reagent and that CX-23 may be a promising lead for developing targeted antihepatocellular carcinoma drugs.

KEYWORDS: CX-23, triptolide (TP), antihepatocellular carcinoma, NAD(P)H:quinone oxidoreductase 1 (NQO1)

Primary liver cancer is one of the most common malignant tumors of the digestive tract in the clinic, 90% of which are hepatocellular carcinoma (HCC). Epidemiological surveys show that approximately 500,000 patients worldwide die of HCC every year, ranking fourth in the world for cancer mortality.¹ For patients with early liver cancer, liver transplantation and resection are the preferred treatments for liver cancer, but most patients have reached late stage or metastasis at the time of diagnosis and cannot meet surgical criteria. For patients who cannot undergo surgery, chemotherapy is one of the important treatments for liver cancer.^{2,3} Chemotherapy can not only control the growth and spread of tumors but also transform inoperable tumors into operable tumors and increase the cancer cure rate. Conventional drugs used for antihepatocellular carcinoma are cytotoxic drugs, among which the more commonly used cytotoxic drugs are doxorubicin, cisplatin, docetaxel, and fluorouracil.⁴ These drugs are more toxic and less effective. Sorafenib is the first-line antihepatocellular carcinoma drug approved by Food and Drug Administration; however, some patients still have treatment failure or tolerability in clinical use. Compared with the control group, the sorafenib treatment group only prolonged the life of the patient for two months.⁵ Therefore, there is urgent need for antihepatocellular carcinoma reagents discovery and development.

Triptolide (TP) is a diterpene triepoxide compound purified from *Tripterygium Wilfordii* Hook F (TWHF)⁶ and possesses the ability to treat diseases such as tumor,⁷ immune system disorders,⁸ diseases of central nervous system,⁹ and cardiovascular.¹⁰ Among the pharmacological effects, its antitumor

activity attracted the most attention of researchers. In vitro studies have shown the high potential antiproliferation of TP against all 60 NCI human tumor cells with the concentration at the nanomolar (nM) levels. Moreover, TP can circumvent tumor drug-resistant tumor cells and sensitize cancer cells to other chemotherapeutic agents.^{11–13} The xeroderma pigmentosum type B (XPB/ERCC3) subunit (ATPase activity) of Transcription factor II Human (TFIIH) was generally accepted the principal TP target. TP can inhibit the ATPase activity of XPB by covalently binding with the thiol of Cys342 of XPB, which initiates transcription by RNA polymerase II (RNAPII) and nucleotide excision repair, offering a consistent mechanism that can be used to interpret most of the known pharmacologic activities of TP.¹⁴ Although TP has great potential as an antitumor agent, its application in the clinic is restricted due to its liver and kidney toxicities.^{15–17} The most common side effects of TP are observed in the liver and kidney, where the toxicities are mainly induced by the accumulation of parent drug.¹⁸

As a great promising an anticancer drug lead, several analogues of TP have been developed (Figure S1). Noteworthy, all the analogues of TP in clinical development adopted prodrug strategies and kept the core structure of TP intact. PG490–88 (omtriptolide), an esterification of 14-OH group of TP by a succinate acid, failed in phase I clinical trial because of its slow and incomplete biotransformation in vivo.¹⁹

Received: September 7, 2018

Accepted: November 27, 2018

Published: November 27, 2018

It indicated the modification of the 14-OH TP impede the enzymatic cleavage due to the hindrance of stereoselectivity. Minnelide is a phosphonooxymethyl derivative of TP and the latest TP analog, which is under phase II clinical trial. Minnelide can be metabolized by alkaline phosphatase and then spontaneously releases TP to carry out the antitumor activity.²⁰ Therefore, simple esterification of 14-OH of TP may not be a good method, but spontaneous transformation is an effective way to achieve the successful goal.

Due to on-target toxicity of TP, it is necessary to develop a cancer targeted pro-drug approach. NAD(P)H:quinone oxidoreductase 1 (NQO1) is a cytosolic reductase and a homodimeric flavoprotein that utilizes NAD(P)H to catalyze the two-electron reduction of a broad range of substrates, most notably quinones.²¹ NQO1 is an attractive anticancer target for its higher levels expressed in many solid tumors.²² In hepatocellular carcinoma, the positive rate and highly positive rate of NQO1 protein were 86.42% and 58.97% in HCC tissues and were significantly higher than in either adjacent nontumor tissues or normal liver tissues. Consistently, the five year survival rates of the cancer patients with overexpressed NQO1 are lower compared with those with low NQO1 levels.^{23,24} Several classes of bioreductive compounds that can undergo NQO1 reduction to active species have been developed.^{25–27} Mitomycin C is present in a clinically used antitumor antibiotic. Other quinones such as β -lapachone, EO9, AZQ, and RH1 that show improved properties over mitomycin have not entered clinical research.²² Alternative strategy is that the antitumor reagents were selectively activated by NQO1 in cancer cells.

We have designed and screened TP analogs that contain quinone propionic acid structure (Figure S2). The resulting complexes were scored using the AutoDock Vina, and the highest scoring compound (CX-23) was selected for further analysis. It can be clearly seen that quinone moiety of CX-23 binds with the residues of the NQO1 active site and flavin adenine dinucleotide (FAD), which is required for NQO1 catalytic activity (Figure 1A). The leaving group triptolide is left outside the substrate binding pocket, suggesting that the interaction of quinone with NQO1 active site is not affected by the introduction of triptolide to quinone.

CX-23 contains a propionic acid, as a linkage between quinone and the C14 hydroxy group, which is the most reactive and chemical modified functional group in TP. Reduction of the quinone propionic acid esters or amides bearing three methyl groups would lead to the corresponding hydroquinones in which severe steric repulsion between three methyl groups promotes rapid lactonization and release of an alcohol or amine. Several quinone propionic acid compounds have been investigated for reductase-activated prodrugs such as those based on mustard and oxindoles for anticancer therapy.²⁸ The quinone can be reduced by NQO1, and the propionic acid can facilitate the spontaneously transformation to release TP (Figure 1C), which can covalently bind to the XPB and exert its pharmacological activities.

To synthesize CX-23, hydroquinone was first converted into CX-21 upon the reaction with methyl β,β -dimethyl acrylate in the presence of methanesulfonic acid. CX-22 was synthesized using NBS and esterified with TP involving dicyclohexylcarbodiimide (DCC) and *N,N*-dimethylaminopyridine (DMAP) to yield CX-23 (Figure 1B). The structures of CX-21, CX-22, and CX-23 were confirmed by mass spectrometry and NMR spectrometry (Figure S3).

Figure 1. (A) Best pose of CX-23 bound NQO1 ($\Delta E = -12.2$ kcal/mol) using AutoDock Vina; the image was visualized using Pymol. Graphical representation of CX-23-NQO1 complex; TP complex is represented as capped sticks. FAD is expressed in blue. (B) Synthesis of CX-23. (C) Proposed metabolic and transformed pathways of CX-23 activated by NQO1.

First, we studied if CX-23 was targeted to XPB. The effect of CX-23 on XPB ATPase activity was evaluated (Figure S4A). CX-23 had no inhibitory effects on the XPB ATPase activity, whereas TP, which had been proved to target the XPB, presented a higher inhibitory, indicating CX-23 cannot directly bind with XPB. Due to the same target for the toxicity and pharmacological activity of TP, the inability of CX-23 to inhibit XPB makes it an ideal prodrug, which can be converted to TP by cellular NQO1 but devoid of undesirable toxicity prior to entry into cells.

We evaluated the effects of CX-23 and TP on the proliferation of HepG2 and normal hepatocytes. CX-23 presents a comparable antitumor cell activity with IC_{50} value of 54.3 nM, and TP retained the considerable antitumor cell activity with IC_{50} value of 43.3 nM (Figure S4B). In contrast, in the case of normal hepatocytes with undetectable levels of NQO1, the IC_{50} value was 540 nM, a value 9.89-fold above its anti-HepG2 activity and 14.3-fold higher than TP with the value of 37.7 nM (Figure S4C), which means a wide therapeutic index for CX-23. The levels of NQO1 activities both in normal hepatocytes and HepG2 cells were studied. The results show that the value of NQO1 activity is 361 ± 84 nmol/min/mg protein in HepG2 cells lysates, but undetected NQO1 activity in normal hepatocyte lysates.

To determine whether CX-23 depended on NQO1-activated for its antiproliferation, we test CX-23 in combination with a NQO1 inhibitor, dicoumarol,²⁹ which alone shows no effect on the HepG2 with the concentration up to 20 μ M. From Figure S4B, we can see that CX-23 shows the antiproliferation of HepG2 when in sole use. However, when in combination with dicoumarol, the antitumor efficacy of CX-23 was partly lost, suggesting that dicoumarol can impede the antiproliferation of HepG2 by CX-23 and that CX-23 may depend on NQO1 to exert its antihepatocellular carcinoma activity.

A complementary assay was used to study the role of NQO1 in the transformation of CX-23 by investigating the metabolism of CX-23 in HepG2 lysates and recombinant NQO1. The metabolism half-life of CX-23 in HepG2 lysates is 7.27 min (Figure S5A), and two major metabolites (TP and CX-21) are identified (Figure 2A). Dicoumarol shows

Figure 2. (A) Metabolism of CX-23 in HepG2 lysates with the presence of NADPH. (B) Metabolism of CX-23 by NQO1 with the presence of dicoumarol. (C) Formation rates of TP in recombinant NQO1 from CX-23. (D) Pharmacokinetics of CX-23 in mice after 1.0 mg/kg ip dose.

concentration-dependent inhibition of CX-23 metabolism in NQO1 and the transformation of CX-23 into TP was completely inhibited with the dicoumarol concentration of 20 μ M (Figure 2B). The recombinant NQO1 can also transform CX-23 into TP and CX-21 in the presence of NADPH with apparent K_m and V_{max} of 0.309 μ M and 0.223 pmol/mg/min, respectively (Figure 2C). Together, the results suggest that NQO1 can facilitate the transformation of CX-23 into TP. The reduction of CX-23 is NQO1-dependent, and the products generated include CX-21 and TP, the latter being capable of inhibit ATPase activity.

To confirm that the antitumor activity of CX-23 was due to the activation by NQO1, we used a panel of primary and cancer cell lines that had high expression of NQO1 to obtain a profile of the general cytotoxicity of CX-23. For human keratinocytes and human skin fibroblast, the average IC_{50} value was above 1.0 μ M. In comparison, the IC_{50} values for HeLa and A549 were 38.2 and 59.4 nM (Figure S4D), respectively, indicating that CX-23 was more selective for the highly expressed NQO1 tumor cells.

Most drugs do not have specific tissue distribution and are easier to be eliminated in plasma and liver. Only a small number of drugs enter the treatment site, which can cause less

effectiveness. In this case, we carry out the metabolic stability of CX-23 both in human plasma and human liver microsomes. After 1 h incubation *in vitro*, the half-life of CX-23 in human plasma (Figure S5B) and liver microsomes (Figure S5C) are above 1 h. The plasma contains abundant carboxyl ester hydrolase and the liver microsomes contains cytochrome P450 enzymes for xenobiotic oxidation, indicating the carboxyl ester hydrolase-resistance of CX-23 and nonoxidized by CYP450 enzymes.

We have set up the analytical method, which showed good linearity, selectivity, and reproducibility, and this method (Tables S1 and S2) can be applied for the pharmacokinetics study of CX-23 (Figure 2D, Table S3). After a single intraperitoneal (ip) dose at 1.0 mg/kg in mice, CX-23 was absorbed quickly with the absorption half-life value of 1.63 min and the time to reach the maximum plasma concentration (t_{max}) is 3 min with a maximum concentration of 243 μ g/L. CX-23 was also distributed quickly with the distribution half-life value of 15.1 min. The ratios of AUC_{liver}/AUC_{plasma} and $AUC_{kidney}/AUC_{plasma}$ are less than 5%, which means less compound exposure in liver and kidney. The plasma concentration of CX-23 was sustained above IC_{50} for 40 min and declined slowly to 1.38 μ g/L at 2 h. After 2 h, the levels of CX-23 in plasma were under detectable. The terminal half-life was 154 min, indicating the parent drug was eliminated quickly without any cumulation *in vivo*.

The *in vivo* antihepatocellular carcinoma activity and toxicity of CX-23 were evaluated by using HepG2 xenograft nude mice model. In a preliminary experiment, we found there is no obvious toxic symptoms of CX-23 after a 1.0 mg/kg ip dose and TP after a 0.2 mg/kg ip dose. Accordingly, 1 week after HepG2 cells inoculated, mice were dosed once daily by ip over a period of 2 weeks. As shown in Figure 3, both TP and CX-23 caused an effective reduction in tumor growth. The equimolar dosage of CX-23 shows the similar antitumor effects (Figure 3A,B) as TP. During the whole treatment period, there are no significant body weight changes (Figure 3C) in the treated animals, suggesting there is no or minimal toxicity.

Figure 3. Antihepatocellular carcinoma activity of CX-23 compared with TP in nude mice. (A) Tumor volume change during the experiment. (B) Tumor weight at the end time point. (C) Mice weight during the experiment. (D) Images of tumor tissues at the end time point. $^{**}p < 0.01$ as compared with control; $n = 8$ mice/group. Student's *t* test was performed for the statistical analysis.

To further determine the toxicity of CX-23, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine, blood urea nitrogen levels were used to evaluate the liver and renal toxicity. As shown in Figure S6, there is no difference for TP and CX-23 group in the levels of ALT and AST, and no pathological abnormalities found in the liver compared with the control group. However, the levels of creatinine and BUN in TP group are significantly increased compared with control group (Figure 4), indicating abnormal renal function. The histological examination (Figure 4B–E) showed that glomerular capillary loop lesions were observed in TP treated group.

Figure 4. Effect of TP/CX-23 on kidney of nude mice. (A) Toxicity evaluation of TP and CX-23 on serum biochemical parameters. Mice were dosed every day for 15 days. * $p < 0.05$, ** $p < 0.01$ vs control. (B) Control group kidney section. (C) Kidney section of TP (0.2 mg/kg)-treated group. (D) Kidney section of CX-23 (0.3 mg/kg)-treated group. (E) Kidney section of CX-23 (1.0 mg/kg)-treated group. Hematoxylin and eosin staining, $\times 200$.

In conclusion, CX-23, a quinone propionic acid conjugated TP and prodrug has shown excellent antihepatocellular carcinoma activity, impeding the tumor cells growth meanwhile reducing the kidney toxicity. CX-23 represents a potent new targeted to NQO1-activated antitumor drugs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.8b00404.

Applied methods, Figures S1–S6, and Table S1–S3 (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: zpengl@nd.edu.

*E-mail: cy101610@qq.com.

ORCID

Zhihong Peng: 0000-0002-2764-4645

Author Contributions

[†]M.L. and W.S. contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The study was supported by Science Foundation for the Youth Scholars of Hubei Provincial Department of Education for Science and Technology Research program (No.: Q2017007).

■ REFERENCES

- (1) Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R. L.; Torre, L. A.; Jemal, A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *Ca-Cancer J. Clin.* **2018**, *68*, 394.
- (2) Inoue, Y.; Tanaka, R.; Fujii, K.; Kawaguchi, N.; Ishii, M.; Masubuchi, S.; Yamamoto, M.; Hirokawa, F.; Hayashi, M.; Uchiyama, K. Surgical Outcome and Hepatic Regeneration after Hepatic Resection for Hepatocellular Carcinoma in Elderly Patients. *Dig. Surg.* **2018**, 1–13.
- (3) Rinninella, E.; Cerrito, L.; Spinelli, I.; Cintoni, M.; Mele, M. C.; Pompili, M.; Gasbarrini, A. Chemotherapy for Hepatocellular Carcinoma: Current Evidence and Future Perspectives. *J. Clin. Transl. Hepatol.* **2017**, *5* (3), 235–248.
- (4) El-Sayyad, H. I.; Ismail, M. F.; Shalaby, F.; Abou-El-Magd, R.; Gaur, R. L.; Fernando, A.; Raj, M. H.; Ouhit, A. Histopathological effects of cisplatin, doxorubicin and 5-fluorouracil (5-FU) on the liver of male albino rats. *Int. J. Biol. Sci.* **2009**, *5* (5), 466.
- (5) Al-Judaibi, B.; Thomas, B.; Wong, P.; Benmassaoud, A.; Chen, J. H.; Dokus, M. K.; Hussaini, T.; Bilodeau, M.; Burak, K. W.; Marotta, P.; Yoshida, E. M. Sofosbuvir-Based Therapy in the Pre-Liver Transplant Setting: The Canadian National Experience. *Ann. Hepatol.* **2018**, *17* (3), 437–443.
- (6) Guo, L.; Duan, L.; Liu, K.; Liu, E. H.; Li, P. Chemical comparison of *Tripterygium wilfordii* and *Tripterygium hypoglaucum* based on quantitative analysis and chemometrics methods. *J. Pharm. Biomed. Anal.* **2014**, *95*, 220–228.
- (7) Brincks, E. L.; Kucaba, T. A.; James, B. R.; Murphy, K. A.; Schwertfeger, K. L.; Sangwan, V.; Banerjee, S.; Saluja, A. K.; Griffith, T. S. Triptolide enhances the tumoricidal activity of TRAIL against renal cell carcinoma. *FEBS J.* **2015**, *282* (24), 4747–4765.
- (8) Grzegorzewska, A. E.; Frankiewicz, D.; Breborowicz, D.; Matlawska, I.; Bylka, W. Disseminated cutaneous Kaposi sarcoma in a patient receiving triptolide/tripdiolide for rheumatoid arthritis. *Med. Sci. Monit.* **2012**, *18* (8), CS67–71.
- (9) Zheng, Y.; Zhang, W. J.; Wang, X. M. Triptolide with potential medicinal value for diseases of the central nervous system. *CNS Neurosci. Ther.* **2013**, *19* (2), 76–82.
- (10) Zhang, Z.; Qu, X.; Ni, Y.; Zhang, K.; Dong, Z.; Yan, X.; Qin, J.; Sun, H.; Ding, Y.; Zhao, P.; Gong, K. Triptolide protects rat heart against pressure overload-induced cardiac fibrosis. *Int. J. Cardiol.* **2013**, *168* (3), 2498–2505.
- (11) Zhu, W.; Li, J.; Wu, S.; Li, S.; Le, L.; Su, X.; Qiu, P.; Hu, H.; Yan, G. Triptolide cooperates with Cisplatin to induce apoptosis in gemcitabine-resistant pancreatic cancer. *Pancreas* **2012**, *41* (7), 1029–1038.
- (12) Chen, Z.; Sangwan, V.; Banerjee, S.; Chugh, R.; Dudeja, V.; Vickers, S. M.; Saluja, A. K. Triptolide sensitizes pancreatic cancer cells to TRAIL-induced activation of the death receptor pathway. *Cancer Lett.* **2014**, *348* (1–2), 156–166.
- (13) Li, Y.; Hu, S. Triptolide sensitizes liver cancer cell lines to chemotherapy in vitro and in vivo. *Panminerva Med.* **2014**, *56* (3), 211–220.
- (14) He, Q. L.; Titov, D. V.; Li, J.; Tan, M.; Ye, Z.; Zhao, Y.; Romo, D.; Liu, J. O. Covalent modification of a cysteine residue in the XPB subunit of the general transcription factor TFIID through single epoxide cleavage of the transcription inhibitor triptolide. *Angew. Chem., Int. Ed.* **2015**, *54* (6), 1859–1863.
- (15) Jin, J.; Sun, X.; Zhao, Z.; Wang, W.; Qiu, Y.; Fu, X.; Huang, M.; Huang, Z. Activation of the farnesoid X receptor attenuates triptolide-induced liver toxicity. *Phytomedicine* **2015**, *22* (10), 894–901.
- (16) Li, X. X.; Du, F. Y.; Liu, H. X.; Ji, J. B.; Xing, J. Investigation of the active components in *Tripterygium wilfordii* leading to its acute hepatotoxicity and nephrotoxicity. *J. Ethnopharmacol.* **2015**, *162*, 238–243.
- (17) Singla, N.; Challana, S. Reproductive toxicity of triptolide in male house rat, *Rattus rattus*. *Sci. World J.* **2014**, *2014*, 879405.
- (18) Zhou, Z. L.; Yang, Y. X.; Ding, J.; Li, Y. C.; Miao, Z. H. Triptolide: structural modifications, structure-activity relationships,

bioactivities, clinical development and mechanisms. *Nat. Prod. Rep.* **2012**, *29* (4), 457–475.

(19) Fidler, J. M.; Li, K.; Chung, C.; Wei, K.; Ross, J. A.; Gao, M.; Rosen, G. D. PG490–88, a derivative of triptolide, causes tumor regression and sensitizes tumors to chemotherapy. *Mol. Cancer Ther.* **2003**, *2* (9), 855–862.

(20) Banerjee, S.; Saluja, A. Minnelide, a novel drug for pancreatic and liver cancer. *Pancreatol.* **2015**, *15* (4 Suppl), S39–43.

(21) Cresteil, T.; Jaiswal, A. K. High levels of expression of the NAD(P)H:quinone oxidoreductase (NQO1) gene in tumor cells compared to normal cells of the same origin. *Biochem. Pharmacol.* **1991**, *42* (5), 1021–1027.

(22) Liao, K.; Niu, F.; Hao, H.-P.; Wang, G.-J. Advances on structure-activity relationship of NQO1-targeting antitumor quinones. *Zhongguo Tianran Yaowu* **2012**, *10* (3), 170–176.

(23) Lin, L.; Sun, J.; Tan, Y.; Li, Z.; Kong, F.; Shen, Y.; Liu, C.; Chen, L. Prognostic implication of NQO1 overexpression in hepatocellular carcinoma. *Hum. Pathol.* **2017**, *69*, 31–37.

(24) Strassburg, A.; Strassburg, C. P.; Manns, M. P.; Tukey, R. H. Differential gene expression of NAD(P)H:quinone oxidoreductase and NRH:quinone oxidoreductase in human hepatocellular and biliary tissue. *Mol. Pharmacol.* **2002**, *61* (2), 320–325.

(25) Bey, E. A.; Bentle, M. S.; Reinicke, K. E.; Dong, Y.; Yang, C.-R.; Girard, L.; Minna, J. D.; Bornmann, W. G.; Gao, J.; Boothman, D. A. An NQO1- and PARP-1-mediated cell death pathway induced in non-small-cell lung cancer cells by β -lapachone. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104* (28), 11832–11837.

(26) Siegel, D.; Yan, C.; Ross, D. NAD (P) H: quinone oxidoreductase 1 (NQO1) in the sensitivity and resistance to antitumor quinones. *Biochem. Pharmacol.* **2012**, *83* (8), 1033–1040.

(27) Huang, X.; Dong, Y.; Bey, E. A.; Kilgore, J. A.; Bair, J. S.; Li, L.-S.; Patel, M.; Parkinson, E.; Wang, Y.; Williams, N. S. An NQO1 substrate with potent anti-tumor activity that selectively kills by PARP1-induced programmed necrosis. *Cancer Res.* **2011**, *71*, 3135–3138.

(28) Volpato, M.; Abou-Zeid, N.; Tanner, R. W.; Glassbrook, L. T.; Taylor, J.; Stratford, I.; Loadman, P. M.; Jaffar, M.; Phillips, R. M. Chemical synthesis and biological evaluation of a NAD (P) H: quinone oxidoreductase-1-targeted tripartite quinone drug delivery system. *Mol. Cancer Ther.* **2007**, *6* (12), 3122–3130.

(29) Tsvetkov, P.; Asher, G.; Reiss, V.; Shaul, Y.; Sachs, L.; Lotem, J. Inhibition of NAD (P) H: quinone oxidoreductase 1 activity and induction of p53 degradation by the natural phenolic compound curcumin. *Proc. Natl. Acad. Sci. U. S. A.* **2005**, *102* (15), 5535–5540.