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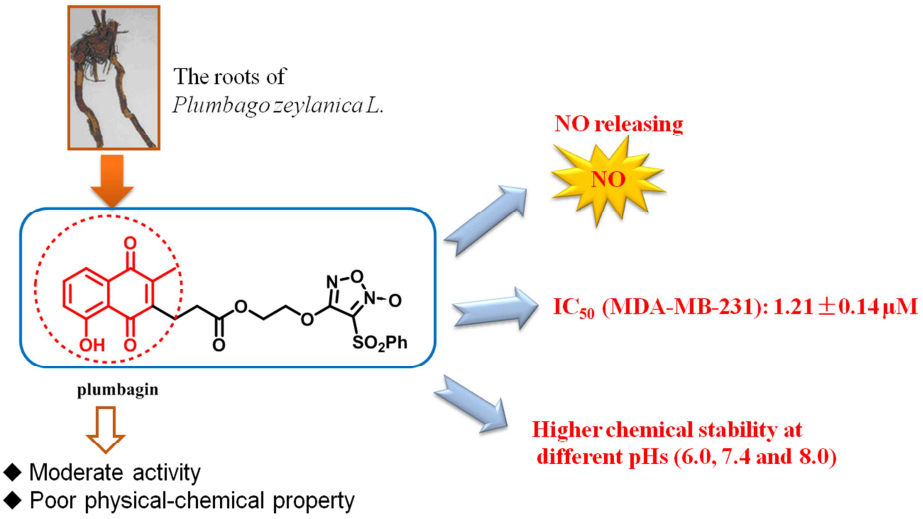

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Novel NO-releasing Plumbagin Derivatives: Design, Synthesis and Evaluation of Antiproliferative Activity

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ABSTRACT

A series of plumbagin/NO donor hybrids were designed, synthesized and evaluated *in vitro* against triple negative breast cancer (MDA-MB-231), hepatocellular (HepG2) and lung (A549) carcinoma cells. Most furoxan-based plumbagin derivatives exhibited significantly superior potency compared to their parent compound. Noticeably, MDA-MB-231 cells are the most sensitive to these furoxan-based plumbagin derivatives as evidenced by IC_{50} values ranging from 1.24 to 5.20 μM. Besides, NO released amounts detection of all hybrids suggested that in most cases, the antiproliferative activities were positively correlated with the levels of intracellular NO release in MDA-MB-231 cells. The most active compound (**11a**) also

possessed higher chemical stability at different pHs (6.0, 7.4 and 8.0) than plumbagin. Together, the above promising results warrant the future potential of plumbagin/NO hybrids as the lead compounds against triple negative breast cancer deserving further research.

Keywords

Plumbagin; NO-releasing; Cytotoxicity; Selectivity; Stability.

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1. Introduction

Triple negative breast cancer (TNBC), which is characterized by the absence of traditional receptors-estrogen receptor (ER), progesterone receptor, and Her-2/neu (ErbB2), represent a very aggressive subtype of breast cancer. Due to its poor prognosis, high incidence of local recurrence and distant metastases, the development of effective anti-TNBC agents, particularly those obtained from natural products has become a hotspot in breast cancer research [1, 2]. Quinones account for one of the largest families of antitumor agents. A large number of natural products as well as synthetic drugs containing quinone chromophor have exerted anticancer effects such as Napabucasin (BBI608), β-lapachone (ARQ761) and STA-21 [3-6], some of them have been successfully developed for clinical use for breast cancer treatment (Fig. 1).

The 1,4-naphthoquinone-based compound, plumbagin (5-hydroxy-2-methyl-1,4-naphthoquinone) was isolated from *Plumbago* species, which have been extensively used for the treatment of rheumatic arthralgia, abscess and scrofula in Traditional Chinese Medicines (TCMs) (Fig. 1) [7]. In recent years, plumbagin has been attracting a rising attention from cancer biologists due to its attractive antitumor activity. Analyses of the sensitivity of the various cancer cell lines indicated that breast, lung, cervical, leukemia and liver cancer cells are sensitive to plumbagin (**1**) [8]. Notably, the naturally occurring plumbagin (**1**) was also found to efficiently induce apoptosis in triple negative breast cancer MDA-MB-231 cells by concomitant downregulation of Bcl-2 expression and NF-κB activity with no effect on MCF-10A cells, the normal breast epithelial cells [9]. However, the development of

plumbagin for cancer therapy was hampered largely by its moderate activity and poor physico-chemical property, especially inferior stability in basic condition, sublimability and penetrating odor etc.. Therefore, it is highly desirable to develop novel chemically stable derivatives of plumbagin to improve its potency without reducing its safety profile .

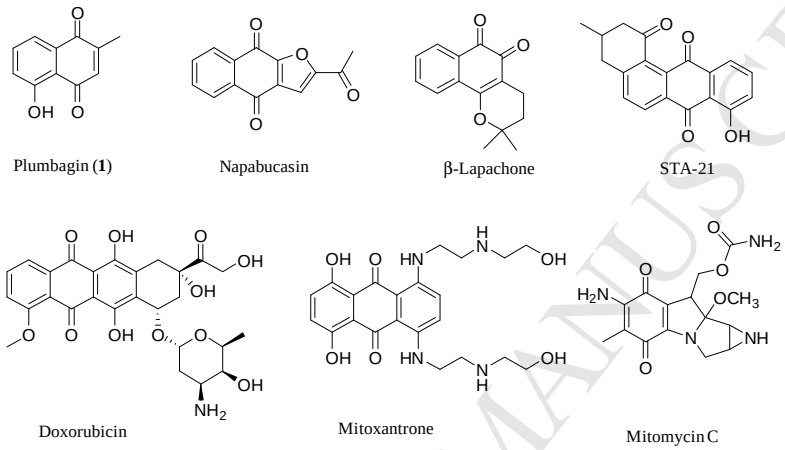

Fig. 1. The structure of plumbagin, and several examples of naphthoquinone-containing natural and synthetic antitumor agents.

Nitric oxide (NO) is a key signaling molecule involved in tumorigenesis. While high level of NO can induce cell cycle arrest and apoptosis, particularly in tumor cells [10]. Indeed, numerous NO-based anti-cancer agents have been developed for the potential application for cancer therapy. Furoxans, an important class of NO donors, are thermally stable and able to produce high level of NO. Their excellent performance makes them attractive for designing antitumor drugs [11]. In the past decade, a variety of promising furoxan-based NO-releasing derivatives have been investigated as anticancer drug candidates [12-14].

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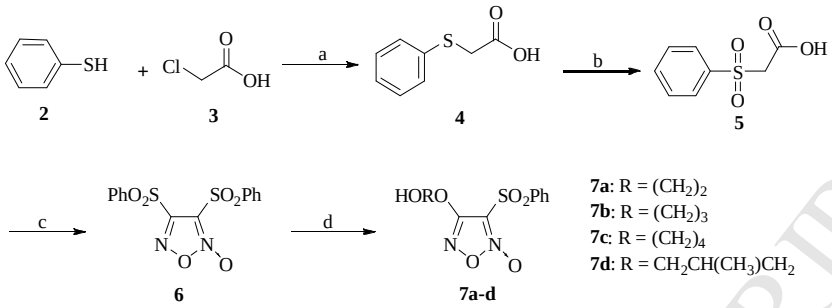
Enlightened by these findings, together with our previous studies on the modification of natural products, we decided to introduce varying carboxylic acid side chains into the C-3 position of the quinone ring, and then designed and synthesized a series of novel plumbagin/NO donor hybrids containing phenylsulfony-substituted furoxans to improve the antiproliferative activity and stability of naturally-occuring plumbagin. Herein, a total of 12 plumbagin/NO donor hybrids (**10a-12d**) have been synthesized with their structure determined by IR, ^{1}H NMR, ^{13}C NMR and ESI/HRMS. The cytotoxic activities of these derivatives on the proliferation of breast cancer (MDA-MB-231) cells, hepatocellular (HepG2) and lung carcinoma (A549) cells were determined by MTT method, respectively. We also detected the NO released amounts of these derivatives by Griess assay to investigate the relationship between NO released amounts and cytotoxic activities.

2. Results and discussion

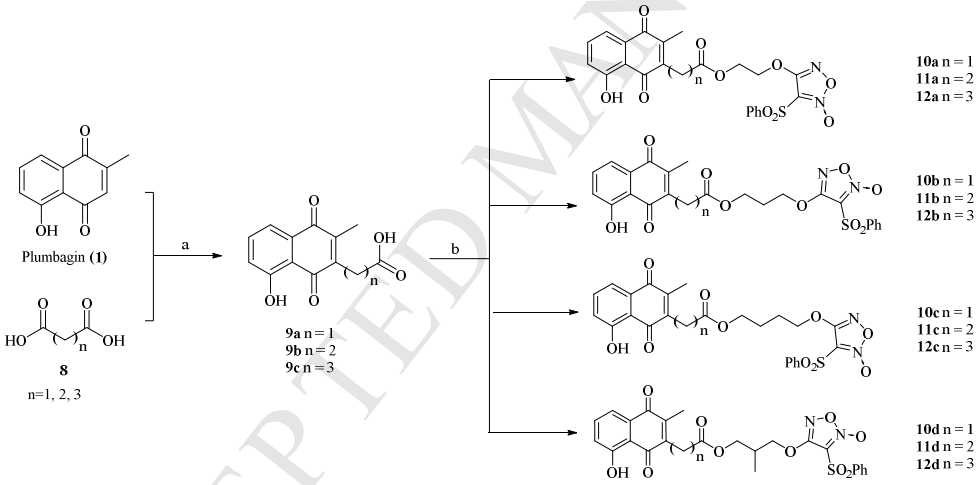
2.1. Chemistry

3,4-Diphenylsulfonyl-furoxan (**6**) was prepared as previously described [15], and the general route is outlined in Scheme 1. The starting material benzenethiol (**2**) was converted to 2-(phenylthio) acetic acid (4) by treatment with chloroacetic acid (**3**). Compound **4** was oxidized by 30% H_2O_2 aqueous solution to give 2- (phenylsulfonyl) acetic acid (**5**) and reacted with fuming HNO_3 to form diphenylsulfonyl-furoxan (**6**). Then the substituted furoxans (**7a-7d**) were obtained from compound **6** with corresponding diols. It is noteworthy that low temperature (-15°C) and low ratio of NaOH (less than

1.5 eq.) are required for preparing substituted furoxans (**7a-7d**). Otherwise, both of phenylsulfonyls would be substituted simultaneously.



Scheme 1. Reagents and conditions (a) NaOH, r.t. 3 h, reflux 1 h (b) H_2O_2, r.t. 3 h (c) fuming HNO_3, 100°C 4 h (d) corresponding diols, 25% NaOH, -15°C 3 h



Scheme 2. Reagents and conditions: (a) $HOOC(CH_2)_nCOOH$ (3.0 eq), silver nitrate (0.3 eq), ammonium persulfate (1.3 eq), aq. 30% CH_3CN, 65-70°C, 3 h (b) **7a-7d**, EDCI/DMAP, dry DCM, 0°C to r.t., 6h

The 1,4-dihydronaphthalenyl carboxylic acids (**9a-9c**) were prepared by combining plumbagin (**1**) with varying length of dicarboxylic acids (**8**) through oxidative decarboxylation and Kochi-Anderson addition following the procedure of Salmon-Chemin et al [16].

The plumbagin/furoxan hybrids (**10a-12d**) were synthesized in moderate to high yields (28%-71%) by condensation of semisynthesized plumbagin analogues (**9a-9c**) with intermediates (**7a-7d**). As shown in Scheme 2.

2.2. In vitro cytotoxic activity

Table 1

Preliminary inhibitory effects of the tested compounds on MDA-MB-231 and HepG2 cells

Cpd.	Inhibition rate (%) at 10 μM*		Cpd.	Inhibition rate (%) at 10 μM*	
	MDA-MB-231	HepG2		MDA-MB-231	HepG2
10a	97.3	93.3	**12a**	97.9	95.1
10b	97.7	97.2	**12b**	95.3	92.7
10c	96.3	87.2	**12c**	92.7	89.2
10d	97.3	96.6	**12d**	94.4	91.5
11a	97.6	95.7	PL	32.8	41.0
11b	96.8	95.1	**9a**	2.4**	11.0**
11c	97.6	95.4	**9b**	5.0**	20.3**
11d	97.4	73.4	**9c**	6.1***	23.2***

* MTT methods, cells were incubated with corresponding compounds at a concentration of 10 μM for 72 h. Values are mean of three independent experiments.

** Inhibition rate (%) at 40 μM.

*** Inhibition rate (%) at 20 μM.

We first detected the preliminary inhibitory effects of plumbagin (**1**) and all the derivatives against two tumor cell lines (MDA-MB-231 and HepG2 cells) by MTT method (Table 1). The antiproliferative activity of the plumbagin derivatives (**9a-9c**) which varying carboxylic acids were introduced to the C-3 position of the quinone ring were inferior compared with the parent compound plumbagin (**1**). Encouragingly,

all plumbagin/NO donor hybrids displayed higher inhibition rate (73.4%-97.9%) than plumbagin (32.8%-41.0%) at a concentration of 10 μM, which inspired us for further assay of cytotoxic activities against MDA-MB-231, HepG2 and A549 cells. Their IC_{50} Values were listed in Table 2. 1,4-naphthoquinone-based anticancer agent Napabucasin and the parent compound plumbagin (PL) were selected as the positive controls.

Almost all plumgabin/NO donor hybrids have exhibited significantly superior potency compared to plumbagin (**1**), among which compound **11a** possessed the highest and selective cytotocixicy against MDA-MB-231 cells with IC_{50} of 1.21 μM despite inferior than the positive control Napabucasin. Further analysis of the above results also concluded that their inhibition effects remained some difference on these three cancer cell lines. Unlike their parent compound (**1**) , nearly all NO hybrids exhibited better selectivities for breast cancer cell (MDA-MB-231) than hepatocellular (HepG2) and lung carcinoma (A549) cells.

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Table 2

Antiproliferative activity of all the tested compounds against three cancer cell lines

Cpd.	Cytotoxicity IC_{50} (μM)			Selectivity ratio IC_{50} (231)/IC_{50} (HepG2)/IC_{50} (A549)	Cpd.	Cytotoxicity IC_{50} (μM)			Selectivity ratio IC_{50} (231)/IC_{50} (HepG2)/IC_{50} (A549)
	MDA-MB-231	HepG2	A549			MDA-MB-231	HepG2	A549	
10a	3.27 ± 0.16	6.11 ± 0.51	18.58 ± 2.0	1: 1.9: 15.7	**11d**	2.67 ± 0.10	6.69 ± 0.08	18.17 ± 1.0	1: 2.5: 6.8
10b	1.76 ± 0.03	4.41 ± 0.12	7.00 ± 0.50	1: 2.5: 4.0	**12a**	1.52 ± 0.11	4.16 ± 0.57	10.25 ± 0.29	1: 2.7: 6.7
10c	5.20 ± 0.13	7.72 ± 0.92	>20	1: 1.5: >3.8.	**12b**	2.42 ± 0.10	4.32 ± 0.50	13.94 ± 1.79	1: 1.8: 5.8
10d	1.51 ± 0.07	3.00 ± 0.19	5.83 ± 0.29	1: 2.0: 3.9	**12c**	1.98 ± 0.28	4.24 ± 0.23	19.64 ± 4.30	1: 2.1: 10.0
11a	1.21 ± 0.14	2.98 ± 0.37	9.31 ± 0.58	1: 2.5: 7.7	**12d**	2.11 ± 0.08	3.74 ± 0.36	12.82 ± 1.33	1: 1.8: 6.1
11b	4.31 ± 0.13	6.79 ± 0.59	>20	1: 1.6: >4.7	PL	14.23 ± 0.93	10.66 ± 1.54	12.40 ± 1.08	1: 0.7: 0.9
11c	1.24 ± 0.10	3.33 ± 0.43	8.31 ± 0.24	1: 2.7: 6.7	Nap[b]	0.34 ± 0.02	0.66 ± 0.16	0.74 ± 0.04	1: 1.8: 2.2

[a] MTT methods, cells were incubated with corresponding compounds for 72 h. IC_{50} (μM) values (means ± SD, n = 3). [b] Nap, Napabucasin (BBI608), as positive control.

Structure and activity relationships (SARs) revealed that mere introduction of carboxylic acids to the C-3 position of the quinone ring (**9a, 9b** and **9c**) could diminish the antiproliferative activities, whereas the hybrids coupling with NO donor moieties in general enhanced the cytotoxicities significantly. Furthermore, various length of the carboxylic acids and diol linkers of theses hybrids had certain differences to potency. Generally, when containing two or three carbons in carboxylic acid linker (n = 2, 3), the compounds with ethoxy- (**11a** and **12a**) and butoxy-furoxan moieties (**11c** and **12c**) manifested a bit higher potency than the propoxy- and 2-methylpropoxy-furoxans (**11b**, **11d**, **12b** and **12d**). Whereas the propoxy and 2-methylpropoxy (**10b** and **10d**) would be favored on the condition of one carbon in carboxylic acid linker (**10a** and **10c**).

2.3. In vitro NO released amounts detection

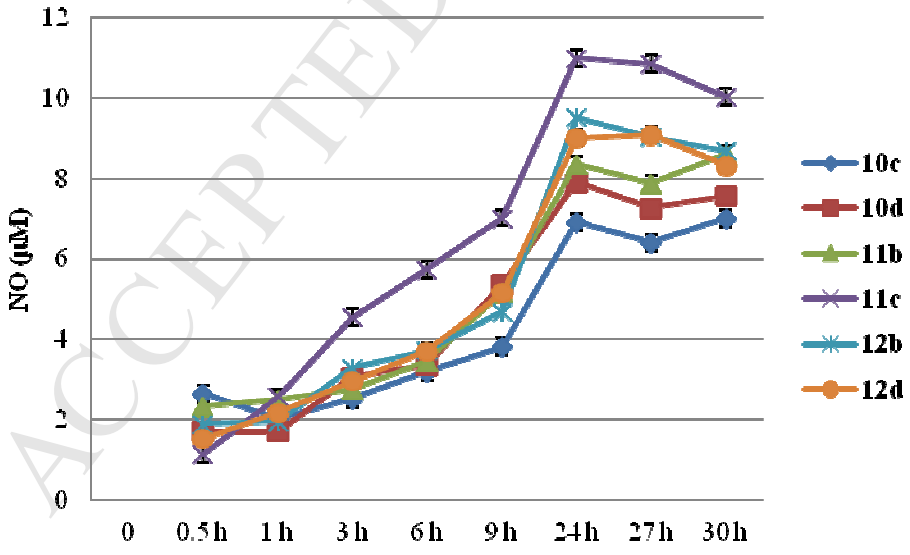

Fig. 2. NO released amounts change curves of partial hybrids producing *vs* time (mean ± SD, n = 3).

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In an effort to test whether the cytotoxic activities were correlated with the concentration of NO produced by these hybrides, we calculated the amounts of nitrate and nitrite, the oxidative end products of NO using the Griess assay according to the standard curve [17]. Then NO amounts change curves of the selected partial hybrids (**10c**, **10d**, **11b**, **11d**, **11c**, **12b** and **12d**) producing with time in MDA-MB-231 cells were shown in Figure 2. NO released amounts of compounds increased with time and reached a high level after 24 h incubation and then maintained steady or peaked off. Therefore, we further decided to evaluate the inhibition rate of all plumbagin/NO donor hybrids at 2 μM and NO production for 24 h to study the correlation between cytotoxic activities and NO released amounts.

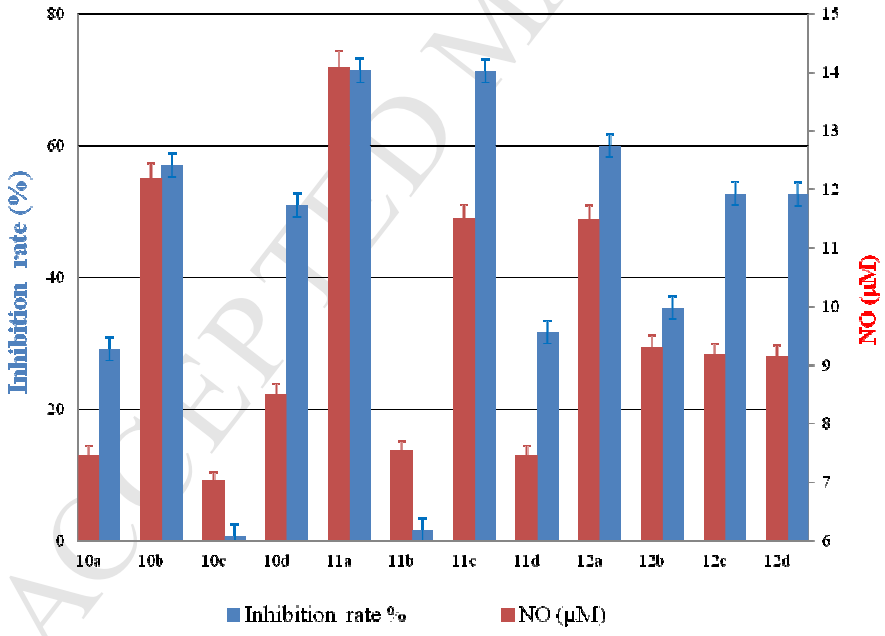

Fig. 3. The correlation between the amounts of NO produced by all these hybrids and cytotoxicities against MDA-MB-231 cells. Cells were cultured at a density of 6.0×10^4/ml, then treated in triplicate (3 × 100 μl) with each compound at 30 μM for 24 h. Cytotoxicities were represented by inhibition rate % at 2 μM.

From the results illustrated from Figure 3, we can observe that the hybrids exhibited NO-releasing properties at levels ranging from 23.5% to 46.9%. And the potent compounds (**10b**, **11a**, **11c** and **12a**) which exhibited high anti-proliferation activity consistently produced high level of NO amounts. Thus, the correlation once again supported the notion that high levels of NO positively exerted cytotoxic activities in human carcinoma cells.

2.4. In vitro chemical stability study

Considering the poor chemical stability of plumbagin, we further decided to select the most active compound (**11a**) using an *in vitro* assay to investigate whether its chemical stability is improved. The stability study was carried out under three conditions (pHs 6.0, 7.4 and 8.0) in order to mimic the acidic duodenum (pH 4.0-6.0), the neutral plasma (pH 7.4) environments and a basic condition (pH 8.0). Data shown in Figure 4 suggested that the stability of plumbagin and compound **11a** in acidic condition were higher than in neutral and basic conditions.

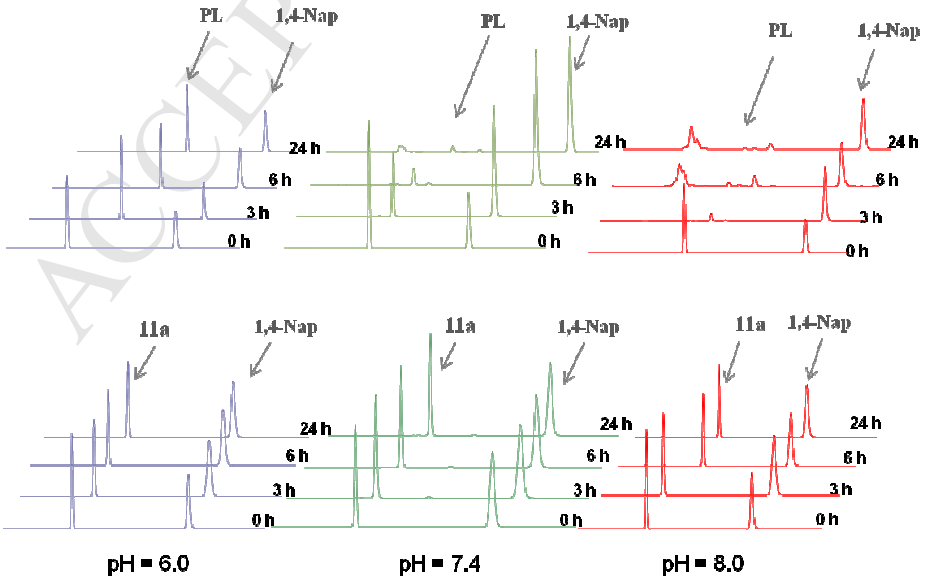

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Fig. 4. *In vitro* chemical stability assay was performed by HPLC method, profile of plumbagin (PL) and compound **11a** in buffer (pH 6.0; 7.4 and 8.0). 1,4-napthoquinone (1,4-Nap) was selected as internal standard.

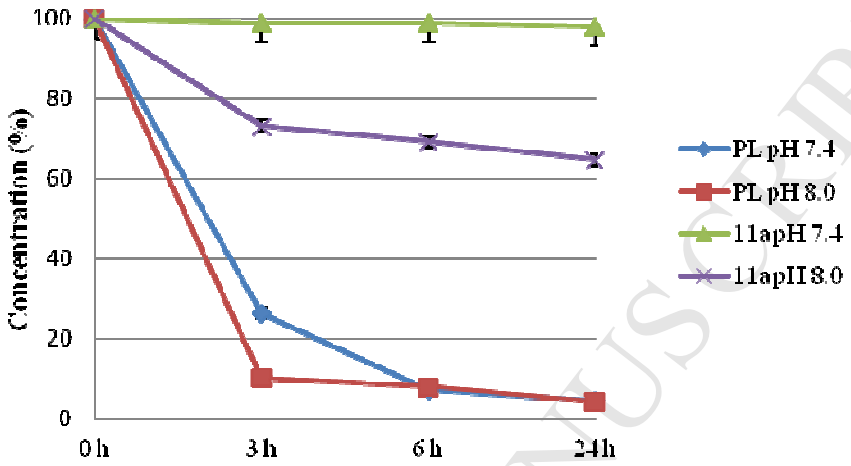

Fig. 5. Concentration–time profiles of plumbagin (PL) and compound **11a** in buffer (pH 7.4, 8.0) (Mean ± SD, n=3).

After being kept in for 24 h at pH 7.4 and 8.0, more than 95% of plumbagin degraded, while in the conditions above, compound **11a** degraded much less than plumbagin at pH 8.0 and still retained more than 98% of the original content after 24 h in pH 7.4 buffer (Figure 5). The presence of hydroxy substituent on quinones is great limitation for chemical stability at basic conditions. According to Borntrager's reaction, it can be postulated that the degradation of plumbagin starts with an attack from the acidic phenolic hydroxyl and then undergoing keto-enol tautomerism (Figure 6). Nevertheless, the plumbagin derivative (**11a**) was much more stable in the basic condition, suggesting that the stability of plumbagin could be enhanced through introducing to C-3 position of the quinone ring by alkyl groups.

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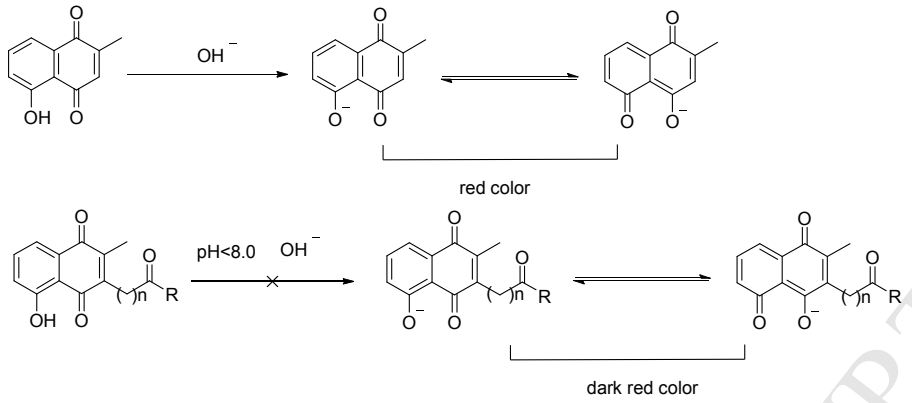

Fig. 6. Chemical structure and possible degradation mechanism of plumbagin, where the phenolic hydroxyl was attacked by OH^- and undergoing keto-enol tautomerism, may play an important role in the chemical instability of plumbagin. While the stability of plumbagin could be enhanced through introducing to C-3 position of the quinone ring by alkyl groups.

3. Conclusion

A total of 12 novel plumbagin/furoxans hybrids have been synthesized and their biological functions were evaluated. Almost all these derivatives displayed improved potency than the parent compound plumbagin, especially against triple negative breast cancer (MDA-MB-231) and hepatocellular (HepG2) cells. SARs revealed that varying length of carboxylic acid linkers and models of the NO donor moiety exerted different cytotoxic effects on the above cell lines. Noticeably, all these hybrids showed greater selectivity against MDA-MB-231 cells compared to HepG2 and A549 cells, which is different from plumbagin, among which **10b, 10d, 11a, 11c** and **12a** have exerted significantly potency with IC_{50} values below 2 μM. NO released amounts measurement by Griess method revealed that the potent compounds which exhibited

high anti-proliferation activities consistently produced high levels of NO in MDA-MB-231, supporting the notion that high levels of NO positively exerted cytotoxic activities in human carcinoma cells. Besides, the most active compound (**11a**) also possess desired chemical stability at different pHs (6.0, 7.4 and 8.0). In conclusion, all these promising results underlined the future potential of plumbagin/furoxans hybrids as lead compounds against triple negative breast cancer.

4. Experimental

4.1. Chemistry

All reagents were purchased from commercial suppliers and used directly unless otherwise stated. CH_2Cl_2 was refluxed over P_2O_5 for an hour and distilled. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE instrument at 25°C. The IR spectra were measured on a fourier transform infrared spectroscopy Nicolet Impact 410. The molecular weights were detected on HP 1100LC/MSD spectrometer.

4.1.1. Isolation and structure identification of plumbagin

Dried and powdered roots (2.2 kg) of *P. zeylamca* were successively and exhaustively extracted with 95% ethanol at 50°C. The ethanol extracted solution was concentrated under reduced pressure to give a dark brown residue. The residue was suspended in water, and then partitioned successively with EtOAc to afford the residues of EtOAc (180 g). The EtOAc soluble-extract was purified by a silica gel column chromatography using petroleum ether (PE): EtOAc = 10:1 (v/v). Orange needle-like crystals of plumbagin were obtained from the first fraction and the purity confirmed

by HPLC was more than 95%. The total yield of plumbagin obtained after extraction and purification by column chromatography was 0.081% (basing on the dried and powdered roots (2.2 Kg) of *P. zeylamca*. ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 11.98 (s, 1H, -OH), 7.63 (dd, *J* = 8.5, 7.5 Hz, 1H, Ar-H), 7.61 (dd, *J* = 7.5, 2.5Hz, 1H, Ar-H), 7.25 (dd, *J* = 8.5, 2.5 Hz, 1H, Ar-H), 6.82 (s, 1H, Ar-H), 2.20 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 190.2, 164.7, 161.1, 149.6, 136.1, 135.4, 131.9, 124.1, 119.2, 115.0, 16.5. MS(ESI) m/z = 187.1 $[M-H]^-$ [18]

4.1.2. Syntheses of intermediates and final compounds

*4.1.2.1 General procedures for the preparation of **9a-9c***

Dicarboxylic acids (3.0 eq), $AgNO_3$ (50.7 mg, 0.3 mmol, 0.3 eq) were successively added to a solution of Plumbagin (188.2 mg, 1 mmol, 1.0 eq) in 30% aqueous acetonitrile (12 ml) at 60℃. when reaction system heated to 70℃, a solution of ammonium persulfate (296.7 mg, 1.3 mmol, 1.3 eq) in 30% aqueous acetonitrile (10 ml) was added dropwise over 1 h, and the resulting solution stirred at 65-70°C for 3-4 h. On cooling, the mixture was extracted with EtOAc (3 × 20 ml), and the organic layer washed with H_2O (3 × 20 ml), dried (Na_2SO_4) and solvent evaporated. The crude material was purified via column chromatography (DCM/MeOH = 20:1) to yield the pure product.

*4.1.2.1.1. 2-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)acetic acid (**9a**)*

Obtained as orange powder (27%); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 11.90 (s, 1H, OH), 7.61 (m, 2H, Ar-H), 7.24 (m, 1H, Ar-H), 3.79 (s, 2H, $C\underline{H}_2$-COOH), 2.22 (s, 3H, CH_3).

^{13}C NMR (75 MHz, $CDCl_3$) δ_C 189.1, 171.1, 160.7, 140.7, 137.2, 132.2, 124.2, 119.2, 32.5, 13.2. MS (ESI) m/z = 269.1 $[M+23]^+$

*4.1.2.1.2. 3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propanoic acid (**9b**)*

Obtained as orange powder (61%); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.09 (s, 1H, OH), 7.59 (m, 2H, Ar-H), 7.22 (m,1H, Ar-H), 2.99 (t, *J* = 7.8 Hz, 2H, C<u>H</u>$_2$–COOH), 2.63 (t, *J* = 7.5 Hz, 2H, C<u>H</u>$_2$-CH_2-CO) 2.22 (s, 3H, CH_3). ^{13}C NMR (75MHz, $CDCl_3$) δ_C 189.6, 177.6, 161.3, 145.9, 144.5, 138.1, 136.1, 124.0 , 119.1 , 32.4, 22.0, 12.9. MS (ESI) m/z = 283.1 $[M+23]^+$

*4.1.2.1.3. 4-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)butanoic acid (**9c**)*

Obtained as orange powder (60%); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.17(s, 1H, OH), 7.61 (m, 2H, Ar-H), 7.25 (m,1H, Ar-H), 2.71 (t, *J* = 7.8 Hz, 2H, CH_2), 2.48 (t, *J* = 7.8 Hz, 2H, CH_2), 2.22 (s, 3H, CH_3), 1.85 (m, 2H, C<u>H</u>$_2$-CH_2-CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 189.8, 184.3, 179.1, 161.2, 145.9, 145.3, 135.9, 132.1, 123.9, 118.9, 114.8, 33.7, 25.6, 23.3, 12.8. MS (ESI) m/z = 297.1 $[M+23]^+$

*4.1.2.2. General procedures for the preparation of **10a-12d***

Compound **9a** (40mg, 0.16 mmol) in 8 ml of CH_2Cl_2 was mixed with N,N-dicyclohexylcarbodie (EDCI, 36 mg, 0.19 mmol) and 4-dimethylamino pyridine (DMAP, 8.6 mg, 0.064 mmol) by stirring at 0°C for 0.5 h, then reacting with corressponding substituted furoxans (68.64 mg, 0.24 mmol) at room temperature for 6

h with strring. The mixture was evaporated *in vacuo* to give the residue, subsequently puried by column chromatography (PE/EA 7:1-5:1 v/v) to afford the title compounds.

4.1.2.2.1.

*4-(2-(2-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)acetoxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**10a**).*

The title compound was obtained starting from **9a** and **7a**.Yellow powder, 31% yield. Analytical data for **10a**: M.p.146-148℃; IR (KBr, cm^{-1}): 3550, 3413, 3131, 1731, 1681, 1661, 1637, 1616, 1552, 1400, 1267, 1165, 1085, 1033, 745, 604; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 11.90 (s, 1H, OH), 8.05 (d, J = 8.0 Hz, 2H, Ar-H), 7.72 (d, J = 7.5 Hz, 1H, Ar-H), 7.63 (m, 4H, Ar-H), 7.24 (m,1H, Ar-H), 4.68 (t, J = 3.7 Hz, 2H, O-CH_2), 4.60 (t, J = 3.7 Hz, 2H, O-CH_2), 3.77 (s, 2H, CH_2-CO), 2.21 (s, 3H, CH_3). ^{13}C NMR (75MHz, DMSO) δ_C 161.3, 136.3, 135.7, 129.7, 128.6, 124.1, 119.4, 114.1, 68.7, 62.0, 22.7, 14.1. ESI/HRMS (m/z) $[M+NH_4]^+$ 532.1025. Calcd for $[C_{23}H_{22}N_3O_{10}S]$: 532.1020.

4.1.2.2.2.

*4-(3-(2-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)acetoxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**10b**).*

The title compound was obtained starting from **9a** and **7b**.Yellow powder, 29% yield. Analytical data for **10b**: M.p.120-121°C; IR (KBr, cm^{-1}): 3552, 3413, 3131, 2360, 2341, 1732, 1664, 1635, 1617, 1555, 1458, 1400, 1159, 745, 604; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 11.83 (s, 1H, OH), 8.0 (d, J = 8.0 Hz, 2H, Ar-H), 7.64 (t, J = 7.5 Hz, 1H, Ar-H), 7.64 (m, 4H, Ar-H), 7.24(m, 1H, Ar-H), 4.50 (t, J = 6.0 Hz, 2H, O-CH_2), 4.35

(t, J = 6.0 Hz, 2H, O-CH_2), 3.71 (s, 2H, CH_2-CO), 2.26 (m, 2H ,O-CH_2-C$\underline{H}_2$), 2.21 (s, 3H, CH_3) ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 169.7, 137.1, 136.5, 130.5 , 128.7 , 124.3, 119.3, 68.4, 61.2, 32.5, 27.6, 13.4. ESI/HRMS (m/z) $[M+NH_4]^+$ 546.1178. Calcd for $[C_{24}H_{24}N_3O_{10}S]$: 546.1777.

4.1.2.2.3.

*4-(4-(2-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)acetoxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**10c**)*

The title compound was obtained starting from **9a** and **7c**. Red waxy solid, 34% yield. Analytical data for **10c**: M.p.85-83°C; IR (KBr, cm^{-1}): 3550, 3413, 3131, 2360, 1721, 1662, 1638, 1618, 1400, 1262, 1166, 601; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 11.96 (s, 1H, OH), 8.07 (d, J = 8.5 Hz, 2H, Ar-H), 7.76 (d, J = 7.5 Hz, 1H, Ar-H), 7.65 (m, 4H, Ar-H), 7.25 (dd, J = 8.0, 1.5 Hz, 1H, Ar-H), 4.46 (t, J = 6.0 Hz, 2H, O-CH_2), 4.26 (t, J = 6.0 Hz, 2H, O-CH_2), 3.74 (s, 2H, CH_2-CO), 2.23 (s, 3H, CH_3), 1.94 (m, 2H, O-CH_2-C$\underline{H}_2$), 1.85(m, 2H, O-CH_2-C$\underline{H}_2$).^{13}C NMR (75 MHz, DMSO) δ_C 136.3, 129.7, 128.6, 124.1, 119.3, 70.9, 64.6, 31.4, 30.2, 24.9. ESI/HRMS (m/z) $[M+NH_4]^+$ 560.1336. Calcd for $[C_{25}H_{26}N_3O_{10}S]$: 560.1333.

4.1.2.2.4.

*4-(3-(2-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)acetoxy)-2-methylpropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**10d**)*

The title compound was obtained starting from **9a** and **7d**. Red solid, 28% yield. Analytical data for **10d**: M.p.72-74°C; IR (KBr,cm^{-1}): 3550, 3413, 3133, 2360, 2341, 1659, 1638, 1618, 1544, 1400, 1323, 1252, 1118, 1026, 609; ^{1}H NMR (300 MHz,

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$CDCl_3$) δ_H 11.81 (s, 1H, OH), 8.04 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.62 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 4.34 (t, *J* = 5.7 Hz, 2H, O-CH_2), 4.21 (t, *J* = 5.7 Hz, 2H, O-CH_2), 3.71 (s, 2H, CH_2-CO), 2.48 (m, 1H, O-CH_2-C<u>H</u>-CH_3), 2.22 (s, 3H, CH_3), 1.14 (d, *J* = 7.0 Hz, 3H, CH-C<u>H</u>$_3$). ^{13}C NMR (75 MHz, DMSO) δ_C 169.7, 161.7, 160.6, 140.2, 137.2, 136.6, 130.5, 128.7, 124.3, 119.3, 71.5, 64.6, 30.8, 29.4, 14.4, 13.5. ESI/HRMS (m/z) $[M+NH_4]^+$ 560.1340. Calcd for $[C_{25}H_{26}N_3O_{10}S]$: 560.1333.

4.1.2.2.5.

*4-(2-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propanoyl)oxy)ethoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**11a**)*

The title compound was obtained starting from **9b** and **7a**. Bright yellow powder, 70% yield. Analytical data for **11a**: M.p.133-136°C; IR (KBr, cm^{-1}): 3551, 3413, 3132, 2360, 2341, 1746, 1637, 1618, 1550, 1460, 1400, 1262, 1165, 609; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.09 (s, 1H, OH), 8.06 (d, *J* = 8.0 Hz, 2H, Ar-H), 7.74 (d, *J* = 7.5 Hz, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 4.62 (t, *J* =3.9 Hz, 2H, O-CH_2), 4.54 (t, *J* = 3.9 Hz, 2H, O-CH_2), 3.00 (t, *J* = 8.0 Hz, 2H, C<u>H</u>$_2$-CO), 2.63 (t, *J* = 8.0 Hz, 2H, C<u>H</u>$_2$-CH_2-CO), 2.24 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO) δ_C 190.0, 184.2, 172.2, 160.6, 159.2, 145.5, 144.9, 137.6, 136.8, 136.5, 132.3, 130.4, 128.7, 124.0, 118.9, 115.1, 69.7, 62.0, 32.3, 22.1, 12.9. ESI/HRMS (m/z) $[M+NH_4]^+$ 546.1183. Calcd for $[C_{24}H_{24}N_3O_{10}S]$: 546.1177.

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4.1.2.2.6.

*4-(3-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propanoyl)oxy)propoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole 2-oxide (**11b**)*

The title compound was obtained starting from **9b** and **7b**. Bright yellow powder, 57% yield. Analytical data for **11b**: M.p.126-128°C; IR (KBr, cm^{-1}): 3412, 3128, 2360, 2342, 1731, 1636, 1615, 1458, 1400, 1260, 1084, 603; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.11 (s, 1H, OH), 8.06 (d, J = 7.9 Hz, 2H, Ar-H), 7.76 (t, J = 7.3 Hz, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 4.50 (t, J = 5.9 Hz, 2H, O-CH_2), 4.30 (t, J = 5.9 Hz, 2H, O-CH_2), 2.97 (t, J = 8.0 Hz, 2H, CH_2-CO), 2.58 (t, J = 8.0 Hz, 2H, CH_2-CH_2-CO), 2.26 (m, 2H, O-CH_2-CH_2), 2.24 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO) δ_C 190.0, 184.3, 172.4, 160.6, 145.4, 145.1, 137.6, 136.9, 136.7, 132.3, 130.5, 128.7, 124.1, 118.9, 115.1, 68.8, 61.0, 32.4, 27.8, 22.1, 12.9. ESI/HRMS (m/z) $[M+NH_4]^+$ 560.1339. Calcd for $[C_{25}H_{26}N_3O_{10}S]$: 560.1333.

4.1.2.2.7.

*4-(4-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)propanoyl)oxy)butoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**11c**)*

The title compound was obtained starting from **9b** and **7c**. Bright yellow powder, 71% yield. Analytical data for **11c**: M.p.122-125°C; IR (KBr, cm^{-1}): 3552, 3413, 3128, 1730, 1636, 1616, 1557, 1459, 1400, 1292, 1258, 1167, 1083, 734, 604; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.12 (s, 1H, OH), 8.06 (d, J = 7.8 Hz, 2H, Ar-H), 7.76 (d, J = 7.2 Hz, 1H, Ar-H), 7.62 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 4.44 (t, J = 5.5 Hz, 2H,

O-CH_2), 4.20 (t, *J* = 5.5 Hz, 2H, O-CH_2), 2.98 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2$-CO), 2.58 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2$-CH_2-CO), 2.23 (s, 3H, CH_3), 1.94 (m, 2H, O-CH_2-C$\underline{H}_2$), 1.84 (m, 2H, O- CH_2-C$\underline{H}_2$). ^{13}C NMR (75 MHz, DMSO) δ_C 190.0, 184.2, 172.4, 160.6, 159.3, 145.4, 145.1, 136.9, 136.6, 132.3, 130.5, 128.7, 124.1, 118.9, 71.5, 64.1, 32.4, 25.1, 24.9, 22.1, 12.9. ESI/HRMS (m/z) $[M+NH_4]^+$ 574.1490. Calcd for $[C_{26}H_{28}N_3O_{10}S]$: 574.1490.

4.1.2.2.8.

*4-(3-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydro-naphthalen-2-yl)propanoyl)oxy)-2-methylpropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**11d**)*

The title compound was obtained starting from **9b** and **7d**. Bright yellow powder, 70% yield. Analytical data for **11d**: M.p.118-120°C; IR (KBr, cm^{-1}): 3409, 3128, 1731, 1685, 1636, 1400, 1293, 1260, 1179, 1167, 603; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.09 (s, 1H, OH), 8.06 (d, *J* = 7.8 Hz, 2H, Ar-H), 7.76 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.62 (m, 4H, Ar-H), 7.22 (m, 1H, Ar-H), 4.37 (t, *J* = 5.0 Hz, 2H, O-CH_2), 4.15 (t, *J* = 5.0 Hz, 2H, O-CH_2), 2.96 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2$-CO), 2.58 (t, *J* = 7.5 Hz, 2H, C$\underline{H}_2$-CH_2-CO), 2.45 (m, 1H, O-CH_2-C$\underline{H}$-CH_3), 2.22 (s, 3H, CH_3), 1.12 (d, *J* = 7.0 Hz, 3H, CH-C$\underline{H}_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 189.7, 184.16, 172.1, 161.3, 158.9, 145.8, 144.7, 136.1, 135.6, 129.7, 128.5, 123.9, 119.0, 72.4, 65.4, 32.6, 29.7, 22.1, 13.6, 12.9. ESI/HRMS (m/z) $[M+NH_4]^+$ 574.1495. Calcd for $[C_{26}H_{28}N_3O_{10}S]$: 574.1490.

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4.1.2.2.9.

*4-(2-((4-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)butanoyl)oxy)eth oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**12a**)*

The title compound was obtained starting from **9c** and **7a**.Yellow powder, 67% yield. Analytical data for **12a**: M.p.132-134°C; IR (KBr, cm^{-1}): 3550, 3413, 3132, 1732, 1637, 1561, 1450, 1400, 1293, 1260, 1166, 1149, 603; ^{1}H NMR (300 MHz, $CDCl_3$) ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.15 (s, 1H, OH) , 8.06 (d, J = 7.5 Hz, 2H, Ar-H), 7.75 (d, J = 7.5 Hz, 1H, Ar-H) , 7.61 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 4.66 (t, J =3.9 Hz, 2H, O-CH_2), 4.59 (t, J =3.9 Hz, 2H, O-CH_2), 2.73 (t, J = 8.0 Hz, 2H, CH_2), 2.52 (t, J = 8.0 Hz, 2H, CH_2), 2.24 (s, 3H, CH_3), 1.91(m, 2H, C$\underline{H_2}$-CH_2-CO). ^{13}C NMR (75MHz, $CDCl_3$) δ_C 189.9, 184.3, 161.2, 145.9, 145.3, 136.0, 135.6, 129.6, 128.6, 123.9, 118.9, 68.9, 61.2, 33.7, 25.6, 23.5, 12.8. ESI/HRMS (m/z) $[M+NH_4]^+$ 560.1339. Calcd for $[C_{25}H_{26}N_3O_{10}S]$: 560.1333.

4.1.2.2.10.

*4-(3-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)butanoyl)oxy)pro poxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**12b**)*

The title compound was obtained starting from **9c** and **7b**. Yellow powder, 66% yield. Analytical data for **12b**: M.p.119-121°C; IR (KBr, cm^{-1}):3550, 3414, 3132, 1732, 1637, 1561, 1450, 1400, 1294, 1261, 1166, 762, 603; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.14 (s, 1H, OH), 8.05 (d, J = 8.0 Hz, 2H, Ar-H), 7.75 (t, J = 7.5 Hz, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.24 (m, 1H, Ar-H), 4.54 (t, J = 6.1 Hz, 2H, O-CH_2), 4.31 (t, J = 6.1 Hz, 2H, O-CH_2), 2.70 (t, J = 8.0 Hz, 2H, CH_2), 2.47 (t, J = 8.0 Hz, 2H, CH_2), 2.26 (m,

2H, O-CH_2-C<u>H</u>$_2$), 2.22 (s, 3H, CH_3), 1.85 (m, 2H, C<u>H</u>$_2$-CH_2-CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 190.0, 184.3, 172.8, 162.2, 158.9, 145.9, 145.3, 138.0, 136.0, 135.6, 132.1, 129.7, 128.5, 123.9, 118.9, 68.1, 60.4, 33.7, 28.0, 25.6, 23.6, 12.8. ESI/HRMS (m/z) $[M+NH_4]^+$ 574.1497. Calcd for $[C_{26}H_{28}N_3O_{10}S]$: 574.1490.

4.1.2.2.11.

*4-(4-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)butanoyl)oxy)but oxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**12c**)*

The title compound was obtained starting from **9c** and **7c**. Yellow powder, 61% yield. Analytical data for **12c**: M.p.112-114°C; IR (KBr, cm^{-1}): 3552, 3477, 3413, 3128, 1723, 1634, 1620, 1567, 1452, 1400, 1295, 1251, 1163, 743, 606; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.17 (s, 1H, OH), 8.07 (d, J = 7.5 Hz, 2H, Ar-H), 7.77 (t, J = 7.0 Hz, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.24 (m,1H, Ar-H), 4.48 (t, J = 6.0 Hz, 2H, O-CH_2), 4.20 (t, J = 6.0 Hz, 2H, O-CH_2), 2.71 (t, J = 7.8 Hz, 2H, -CH_2), 2.46 (t, J = 7.8 Hz, 2H, CH_2), 2.22 (s, 3H, CH_3), 2.00-1.86 (m, 4H, O-CH_2-C<u>H</u>$_2$-C<u>H</u>$_2$), 1.86 (m, 2H, C<u>H</u>$_2$-CH_2-CO). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 190.0, 184.3, 173.0, 161.2, 158.9, 145.9, 145.3, 136.0, 135.6, 132.1, 129.6, 128.6, 123.9, 118.9, 70.9, 63.7, 33.8, 25.7, 25.3, 25.0, 23.6, 12.8. ESI/HRMS (m/z) $[M+NH_4]^+$ 588.1649. Calcd for $[C_{27}H_{30}N_3O_{10}S]$: 588.1646.

4.1.2.2.12.

*4-(3-((3-(8-hydroxy-3-methyl-1,4-dioxo-1,4-dihydronaphthalen-2-yl)butanoyl)oxy)-2-methylpropoxy)-3-(phenylsulfonyl)-1,2,5-oxadiazole-2-oxide (**12d**)*

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The title compound was obtained starting from **9c** and **7d**. Yellow powder, 69% yield. Analytical data for **12d**: M.p.107-110°C; IR (KBr, cm^{-1}): 3551, 3413, 3128, 2360, 1723, 1716, 1620, 1567, 1452, 1400, 1259, 1163, 743, 607; ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 12.14 (s, 1H, OH), 8.06 (d, *J* = 7.5 Hz, 2H, Ar-H), 7.75 (t, *J* = 7.5 Hz, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.23 (m, 1H, Ar-H), 4.38 (t, *J* = 6.0 Hz, 2H, O-CH_2), 4.17 (t, *J* = 6.0 Hz, 2H, O-CH_2), 2.70 (t, *J* = 7.8 Hz, 2H, CH_2), 2.47 (t, *J* = 7.8 Hz, 2H, CH_2), 2.45 (m, 1H, O-CH_2-C<u>H</u>-CH_3), 2.21 (s, 3H, CH_3), 1.85 (m, 2H, C<u>H</u>$_2$-CH_2-CO), 1.14 (d, *J* = 7.0 Hz, 3H, CH–C<u>H</u>$_3$). ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 190.0, 184.3, 172.8, 161.2, 159.0, 145.9, 145.3, 136.0, 135.6, 129.7, 128.5, 123.9, 118.9, 72.5, 65.1, 33.7, 32.7, 25.6, 23.6, 13.6, 12.8. ESI/HRMS (m/z) $[M+NH_4]^+$ 588.1644. Calcd for $[C_{27}H_{30}N_3O_{10}S]$: 588.1646.

4.2. Biological Experiments.

4.2.1. Cytotoxic assay in vitro

All Plumbagin/NO hybrids were evaluated for their cytotoxic activities against breast carcinoma cells (MDA-MB-231), hepatocellular carcinoma cells (HepG2) and lung carcinoma cells (A549) by MTT method. Briefly, 6.0×10^4/ml (MDA-MB-231 cells), 5.0×10^4/ml (HepG2 and A549 cells), respectively, were seeded into 96-well plates and allowed to adhere for 24 h. Each compounds was dissolved in DMSO and diluted to 10 mM. After incubated in 37°C CO_2 incubator for 72 h, MTT solution (5 mg/ml) was added, and the plate was further incubated in 37°C CO_2 incubator for another 4 h. Abandoned supernatant before adding 100 μl DMSO to each well. Optical density (OD) was measured using an enzyme-linked immunosorbent assay (ELISA) reader at

570 nm. The number of viable cells was determined from the absorbance. Assays were performed in triplicate wells. Data are presented as the mean ± SD (n = 3).

4.2.2. NO released amounts detection

All hybrides were evaluated for their NO released amounts by Griess method. Specifically, MDA-MB-231 cells were cultured at a density of 6.0×10^4/ml, then treated in triplicate (3×100 μl) treated with each compound at 30 μM (3×10 μl) for different time, and then the contents of nitrate/nitrite in the cell lysates were determined by Griess assay. The individual values were determined by measuring absorbance at 540 nm and calculated according to the standard curve. Data were mean of three independent experiments.

4.2.3. In vitro stability study

In vitro chemical stability assay was performed by HPLC method. 1,4-Napthoquinone was selected as internal standard. Compounds PL/1,4-napthoquinone, **11a**/1,4-napthoquinone were separated using a Phenomenex Luna reverse-phase C_{18} (2)-HTS column. The mobile phase was 30:70 (water: methanol, v/v) and 20:80 (water: methanol, v/v), respectively. The injection volume was 5 μl and the flow rate was 1 ml/min. The detector was set at 254 nm. For hydrolysis, compound PL, **11a** and 1,4-napthoquinone were diluted in methanol at 1000 μM, respectively. Then these solution were diluted to appropriate concentration using three different PBS buffer (phosphate-buffered saline) to provide the following pHs: 6.0; 7.4 and 8.0. During the assay, all samples were maintained at 37°C. Aliquots were taken from the solution at the following times: 0, 3, 6 and 24 h. All analyses were conducted in triplicate, and

the results were expressed as the averages of the concentrations in percentages (± standard error of the mean [SEM]).

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Figure and Scheme Captions

Fig. 1. The structure of plumbagin, and several examples of naphthoquinone-containing natural and synthetic antitumor agents.

Fig. 2. NO released amounts change curves of partial hybrids producing *vs* time (mean ± SD, n = 3).

Fig. 3. The correlation between the amounts of NO produced by all these hybrids and cytotoxicities against MDA-MB-231 cells. Cells were cultured at a density of 6.0×10^4/ml, then treated in triplicate (3 × 100 μl) with each compound at 30 μM for 24 h. Cytotoxicities were represented by inhibition rate % at 2 μM.

Fig. 4. *In vitro* chemical stability assay was performed by HPLC method, profile of plumbagin (PL) and compound **11a** in buffer (pH 6.0; 7.4 and 8.0). 1,4-napthoquinone (1,4-Nap) was selected as internal standard.

Fig. 5. Concentration–time profiles of plumbagin (PL) and compound **11a** in buffer (pH 7.4, 8.0) (Mean± SD, n=3).

Fig. 6. Chemical structure and possible degradation mechanism of plumbagin, where the phenolic hydroxyl was attacked by OH^- and undergoing keto-enol tautomerism, may play an important role in the chemical instability of plumbagin. While the stability of plumbagin could be enhanced through introducing to C-3 position of the quinone ring by alkyl groups.

Scheme 1. Reagents and conditions (a) NaOH, r.t. 3 h, reflux 1 h (b) H_2O_2, r.t. 3 h (c) fuming HNO_3, 100°C 4 h (d) linker with two hydroxyl groups, 25% NaOH, -15°C 3 h

Scheme 2. Reagents and conditions: (a) $HOOC(CH_2)_nCOOH$ (3.0 eq), silver nitrate

(0.3 eq), ammonium persulfate (1.3 eq), aq. 30% CH_3CN, 65-70°C, 3 h (b) **7a-7d**, EDCI/DMAP, dry DCM, 0°C to r.t., 6h

Table 1. Preliminary inhibitory effects of the tested compounds on MDA-MB-231 and HepG2 cells. * MTT methods, cells were incubated with corresponding compounds at a concentration of 10 μM for 72h. Values are mean of three independent experiments. ** Inhibition rate (%) at 40 μM. *** Inhibition rate (%) at 20 μM.

Table 2. Antiproliferative activity of all the tested compounds against three cancer cell lines. [a] MTT methods, cells were incubated with corresponding compound for 72 h. IC_{50} (μM) values (means ± SD, n = 3). [b] Nap, Napabucasin (BBI608), as positive control.

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Highlights

- A series of NO-releasing plumbagin (PL) derivatives have been designed and synthesized.
- Their anti-tumor activity against MDA-MB-231, HepG2 and A549 cells *in vitro* were evaluated. MDA-MB-231 cells are the most sensitive cells.
- The antiproliferative activities positively correlated with the levels of intracellular NO release in MDA-MB-231 cells.
- Compound **11a** possessed higher chemical stability at different pHs (6.0, 7.4 and 8.0) than plumbagin.

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