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L-Proline catalyzed three-component synthesis of *para*-naphthoquinone-4-aza-podophyllotoxin hybrids as potent antitumor agents

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ABSTRACT A series of novel *para*-naphthoquinone embodied 4-aza-podophyllotoxin hybrids, designed *via* molecular hybridization approach, were synthesized in very good yields using one-pot condensation of 3,4-methylenedioxyaniline, aldehydes and 2-hydroxy-1,4-naphthoquinone in the presence of L-proline. All the synthetic derivatives were fully characterized by spectral data and evaluated for their antitumor activity on human hepatoma cells (HepG2) and Henrietta Lacks strain of cancer cells (Hela). Among the 18 new compounds screened, 12-(3,4,5-trimethoxyphenyl)-5,10-dihydro-benzo[*i*][1,3]dioxolo[4,5-*b*]acridine-6,11-dione (**4o**) has pronounced activity. The results demonstrated potential importance of molecular hybridization in the development of **4o** as potential antitumor agent.

Keywords: L-Proline; Aldehydes; *para*-Naphthoquinones; Antitumor; Hybrids

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Introduction

Naturally occurring *para*-naphthoquinones and their analogues, having wide spectrum of activities such as anticancer,[1] antifungal,[2] antibacterial,[3] antiviral,[4] anti-inflammatory,[5] antimalaria,[6] antiplatelet,[7] antithrombotic,[8] antiallergic,[9] apoptotic[10] and lipoxygenase inhibiting,[11] are frequently used by the researchers to develop novel synthetic and semisynthetic *para*-naphthoquinones based therapeutic agents.[12] Furthermore, *para*-naphthoquinones have also been shown to inhibit human DNA topoisomerase.[13] A number of *para*-naphthoquinone derivatives having nitrogen atom received a great deal of attention for their anticancer activity.[14] Therefore, the development of facile approaches to access these novel targets with structural diversity is highly desirable and valuable for medicinal chemistry and drug discovery.

Podophyllotoxin, a natural lignan, is a good inhibitor of tubulin polymerization with antitumoral properties but it is too toxic for therapeutic use. In order to obtain less toxic drugs, many heterolignans have been prepared. 4-Aza-podophyllotoxin, a structural modification of podophyllotoxin, was reported to retain a most of the cytotoxicity associated with the parent lignan, and could markedly inhibit tubulin polymerization.[15] Therefore, the development of more potent, specific and cell permeable 4-aza-podophyllotoxin analogs with enhanced antiproliferative and tubulin polymerization properties has been carried out in recent years with encouraging results.[16]

Over the last few years, molecular hybridization strategy has emerged as an effective direction in modern medicinal chemistry for the exploration of novel and highly active compounds. In many cases, hybrids are able to improve their precursors' properties, to overcome resistance associated to individual fragments or to show different activities or even different mechanisms of action to that of their precursors.[17] We therefore envisaged that integrating natural *para*-naphthoquinone and pharmacophoric 4-aza-podophyllotoxin moieties in one molecular platform could potentially produce novel compounds with significant synergistic antitumor properties. These hybrid moleculesare

proposed to target the dual inhibition of DNA topoisomerase and tubulin polymerization (Figure 1)

< Figure 1>

These new *para*-naphthoquinone-4-aza-podophyllotoxin hybrids (**4a**–**r**) were prepared *via* one-pot three-component cyclocondensation reaction between 3,4-methylenedioxyaniline, aldehydes and 2- hydroxy-1,4-naphthoquinone in ethanol containing a catalytic amount of L-proline.

< Scheme 1>

Results and Discussion

In the initial investigation, 3,4-methylenedioxyaniline, benzaldehyde and 2-hydroxy-1,4-naphthoquinone were chosen as model reagents for the screening of reaction conditions. The results are summarized in Table 1. The reaction was first carried out in ethanol in the absence and presence of several additives. It was found that only 46% of the target compound **4a** was obtained in the absence of additive (Table 1, entry 1). Some proton acids (Table 1, entries 2-4), Lewis acids (Table 1, entries 5 -6), bases (Table 1, entries 7-8) and other amino acids (Table 1, entries 9-10) can catalyze this reaction with moderate yields. The best result was obtained when L-proline was used according to the yield and the reaction time (Table 1, entry 11). So L-proline was chosen as the catalyst for this reaction. We also evaluated the amount of L-proline required for this reaction. The results from Table 1 (entries 11-14) show that 10 mol % L-proline in refluxing ethanol is sufficient to initiate the reaction. Higher loading of the catalyst had no significant influence on the reaction yield.

<Table 1>

Based on the optimized reaction conditions, a series of novel *para*-naphthoquinone embodied 4-aza-podophyllotoxin hybrids were synthesized. The results, summarized in Table 2, show that the three-component reaction of 3,4-methylenedioxyaniline, aldehydes and 2-hydroxy-1,4-naphthoquinone in the presence of L-proline in refluxing ethanol gave the corresponding products in

moderate to good yields. This methodology can be applied to aromatic aldehydes either with electron-withdrawing groups (such as, nitro and halogen) or electron-donating groups (such as, methoxy and methyl) with excellent yields under the same conditions. Therefore, we conclude that the electronic nature of substituents of the aromatic aldehyde had no significant effect on the reaction. Even the heterocyclic aldehyde could be used in this reaction (Table 2, entries 16-17). In addition, an aliphatic aldehyde (Table 2, entry 18) also showed high reactivity under this standard condition providing corresponding 12-methyl-5,10-dihydro-benzo[*i*][1,3]dioxolo[4,5-*b*]acridine-6,11-dione in a good yield of 72%. In all cases, the resulting novel *para*-naphthoquinone embodied 4-aza-podophyllotoxin hybrids as the reaction mixtures are allowed to cool to room temperature and are isolated by simple filtration followed by recrystallized from ethanol to afford the pure compounds. All the synthesized compounds were characterized by ^{1}H NMR, ^{13}C NMR and element analysis. Spectral data of all synthesized hybrid compounds were in good agreement with the proposed structures. IR spectrum revealed the presence of NH bond (3285-3352 cm^{-1}), C=O (1630-1681 cm^{-1}) functional groups in the synthesized hybrid compounds. This was further confirmed by ^{1}H NMR spectrum, which displayed two additional singlets around δ = 5-6 and d 9-10 ppm for chiral CH and NH in dihydropyridine ring respectively. In addition, the ^{13}C NMR spectrum of **4** showed characteristic signals around δ = 30-40 ppm (due to the R–CH group), 180-181 ppm (arising from the two nonequivalent carbonyl groups). The molecular formula of all the synthesized compounds was confirmed by element analysis. The purity of the compounds was ascertained by HPLC and was found to be >97% pure.

<Table 2>

A plausible mechanism for the regioselective formation of products **4** is shown in Scheme 2. We suggest that that the reaction of L-proline with the aldehyde results in the intermediary iminium ion **A**.

The higher reactivity of the iminium ion condense with 2-hydroxy-1,4-naphthoquinone to form the ene dione intermediate **B**, which undergoes Michael addition with 3,4-methylenedioxyaniline to form intermediate **C**. This step is then followed by cyclization and dehydration to yield to product **4**.

<Scheme 2>

The biological activities of this series of *para*-naphthoquinone-4-aza-podophyllotoxin hybrids were evaluated by an cytotoxicity assay, which was carried out in a panel of two human tumor cell lines (liver and ovarian) by using the MTT method. The results are summarized in Table 3, and compared to 4-aza-podophyllotoxin (4-AP). It is observed that most of the compounds are significantly cytotoxic. Among the 18 new compounds screened, **4o** has pronounced activity. The results in Table 3 showed also some important structure-activity relationships (SARs) for this series of derivatives. First, the *para*-quinone and dihydroacridine moieties appeared to have an important effect upon cytotoxicity, quinone reduction or the aromatization of dihydroacridine was less potent cytotoxicity, than the corresponding analogues with a *para*-quinone or dihydroacridine moiety. Secondly, the wide activity range observed for compounds **4a-4r** indicated that the nature of substituents at the C-12 position markedly affected the activity profile of these compounds. It appears from the data that 4-methoxy substitutions in the 12-phenyl ring enhances the cytotoxicity as seen in compounds **4c** and **4o** for the two cancer cell line. No substituent in the 12-phenyl ring also enhance the cytotoxicity. It is worthwhile to note that all these compounds have lesser cytotoxicity on non-cancerous L02 cells. The results demonstrated potential importance of molecular hybridization in the development of **4o** as a potential antitumor agent.

<Table 3>

Conclusion

In summary, we have developed an efficient, clean and environmentally friendly procedure to generate

para-naphthoquinone-4-aza-podophyllotoxin hybrids *via* the L-proline catalyed three-component condensation of 3,4-methylenedioxyaniline, aldehydes and 2-hydroxy-1,4- naphthoquinone. Moreover, the cytotoxic activities of these compounds were evaluated *in vitro* on two different cancer cell lines, and the results show that some compounds exhibited excellent antitumor activities against HepG2 and Hela.

Experimental

General

IR spectra were determined on FTS-40 infrared spectrometer. NMR spectra were determined on Bruker AV-400 spectrometer at room temperature using TMS as internal standard. Chemical shifts (d) are given in ppm and coupling constants (*J*) in Hz. Elemental analysis was performed by a Vario-III elemental analyzer. Melting points were determined on a XT-4 binocular microscope and were uncorrected. Commercially available reagents were used throughout without further purification unless otherwise stated.

General procedure for the synthesis of compounds 4

To a mixture of 3,4-methylenedioxyaniline (1 mmol), aldehyde (1 mmol), 2-hydroxy-1,4-naphthoquinone (1 mmol) and ethanol 5 mL), L-proline (0.1 mmol) was added. The mixture was stirred at reflux for an appropriate time (Table 2). After completion of the reaction (TLC), the reaction mixture was cooled to room temperature. The precipitate was collected by filtration and purified by recrystallization from ethanol to afford the pure product **4**.

12-Phenyl-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4a**): blue power, m.p. >300 ^{o}C; IR (KBr): *v* 3338, 3026, 2896, 1666, 1626, 1609, 1520, 1505, 1484, 1365, 1245, 1219, 1040, 723, 532 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.94 (s, 1H), 8.02 (d, 1H, *J* = 7.2 Hz, ArH), 7.88 (d, 1H, *J* = 7.2 Hz, ArH), 7.81-7.71 (m, 2H, ArH), 7.26-7.08 (m, 6H, ArH), 6.82 (s, 1H, ArH),

5.93 (d, 2H, J = 24.4 Hz, OCH_2O), 5.35 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.9, 147.9, 147, 144.6, 140.1, 135.3, 133.1, 133.0, 130.8, 130.0, 128.9, 127.6, 126.7, 126.2, 125.9, 117.8, 112.2, 109.2, 101.6, 98.8, 37.5; Anal. Calc. for $C_{24}H_{15}NO_4$: C 75.58, H 3.96, N 3.67; found: C 75.65, H 3.99, N 3.71.

12-(4-Methylphenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4b**): deongaree power, m.p. >300 ^oC; IR (KBr): v 3321, 3080, 2879, 1667, 1630, 1611, 1508, 1482, 1353, 1241, 1221, 1043, 725 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.87 (s, 1H), 8.01 (d, 1H, J = 7.2 Hz, ArH), 7.88 (d, 1H, J = 7.2 Hz, ArH), 7.80-7.70 (m, 2H, ArH), 7.12-6.98 (m, 5H, ArH), 6.77 (s, 1H, ArH), 5.92 (d, 2H, J = 22.8 Hz, OCH_2O), 5.30 (s, 1H, CH), 2.16 (s, 1H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.9, 146.9, 145.1, 144.6, 140.0, 135.7, 135.3, 133.1, 133.0, 130.8, 130.0, 129.4, 127.5, 126.2, 125.8, 118.0, 112.5, 109.2, 101.6, 98.8, 37.9, 21.0; Anal. Calc. for $C_{25}H_{17}NO_4$: C 75.94, H 4.33, N 3.54; found: C 76.02, H 4.31, N 3.59.

12-(4-Methoxylphenyl)-5,10-dihydro-benzo[*i*][1,3]dioxolo[4,5-*b*]acridine-6,11-dione (**4c**): deongaree power, m.p. 273-274 ^oC; IR (KBr): v 3285, 3028, 2903, 1670, 1638, 1608, 1507, 1481, 1361, 1242, 1220, 1035, 726 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.98 (s, 1H), 8.01 (d, 1H, J = 7.6 Hz, ArH), 7.88 (d, 1H, J = 7.6 Hz, ArH), 7.81-7.72 (m, 2H, ArH), 7.13 (d, 1H, J = 7.6 Hz, ArH), 7.07 (s, 1H, ArH), 6.78-6.74 (m, 3H, ArH), 5.92 (d, 2H, J = 21.4 Hz, OCH_2O), 5.30 (s, 1H, CH), 3.63 (s, 1H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.9, 158.1, 146.9, 144.6, 140.3, 139.8, 135.3,133.1, 133.0, 130.8, 130.0, 128.7, 126.2, 125.8, 118.1, 114.2, 112.6, 109.2, 101.6, 98.8, 55.4, 37.8; Anal. Calc. for $C_{25}H_{17}NO_4$: C 72.99, H 4.16, N 3.40; found: C 73.05, H 4.12, N 3.47.

12-(3-Methoxylphenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4d**): deongaree power, m.p. 275-276 ^oC; IR (KBr): v 3343, 3050, 2899, 2833, 1667, 1606, 1592, 1503, 1486, 1366, 1263, 1240, 1223, 1037, 725 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.91 (s, 1H), 8.02 (d, 1H,

J = 7.2 Hz, ArH), 7.89 (d, 1H, *J* = 7.6 Hz, ArH), 7.81-7.71 (m, 2H, ArH), 7.12-7.07 (m, 2H, ArH), 6.83-6.77 (m, 3H, ArH), 6.68-6.66 (m, 1H, ArH), 5.91 (d, 2H, *J* = 23.2 Hz, OCH_2O), 5.32 (s, 1H, CH), 3.66 (s, 1H, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.9, 159.7, 149.4, 147.0, 144.6, 140.1, 135.3, 133.1, 133.0, 130.8, 130.0, 129.9, 126.2, 125.9, 120.0, 117.7, 114.0, 112.2, 111.5, 109.2, 101.6, 98.9, 55.4, 36.9; Anal. Calc. for $C_{25}H_{17}NO_4$: C 72.99, H 4.16, N 3.40; found: C 73.01, H 4.19, N 3.49.

12-(4-Chorophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4e**): blue power,, m.p. 300 °C; IR (KBr): *v* 3337, 3082, 2899, 1665, 1638, 1610, 1523, 1505, 1484, 1364, 1243, 1040, 724, 535 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H), 8.02-7.71 (m, 6H, ArH), 7.55 (d, 1H, *J* = 8.4 Hz, ArH), 7.36 (d, 1H, *J* = 10.4 Hz, ArH), 7.08 (s, 1H, ArH), 6.81 (s, 1H, ArH), 5.92 (d, 2H, *J* = 22.4 Hz, OCH_2O), 5.37 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.1, 180.8, 151.4, 147.1, 144.7, 140.2, 136.5, 135.3, 133.0, 129.6, 129.2, 128.9, 128.8, 125.9, 117.0, 118.0, 109.1, 105.6, 1031, 101.7, 98.9, 37.4; Anal. Calc. for $C_{24}H_{14}ClNO_4$: C 69.32, H 3.39, N 3.37; found: C 69.26, H 3.42, N 3.29.

12-(2-Chorophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4f**): deongaree power, m.p. 260-261 °C; IR (KBr): *v* 3336, 3079, 29.4, 1666, 1632, 1613, 1525, 1505, 1483, 1385, 1338, 1241, 1221, 1039, 725 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.92 (s, 1H), 8.01 (d, 1H, *J* = 7.2 Hz, ArH), 7.83-7.71 (m, 1H, ArH), 7.36 (d, 1H, *J* = 7.2 Hz, ArH), 7.29 (d, 1H, *J* = 7.2 Hz, ArH), 7.15-7.12 (m, 2H, ArH), 7.04 (s, 1H, ArH), 6.62 (s, 1H, ArH), 5.90 (d, 2H, *J* = 26.0 Hz, OCH_2O), 5.83 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.7, 180.6, 147.2, 146.0, 144.6, 140.6, 135.3, 133.0, 132.9, 130.9, 130.8, 130.7, 129.6, 128.3, 126.2, 125.8, 117.3, 111.8, 107.9, 101.7, 99.2, 37.9; Anal. Calc. for $C_{24}H_{14}ClNO_4$: C 69.32, H 3.39, N 3.37; found: C 69.37, H 3.40, N 3.41.

12-(4-Fluorophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4g**): blue power,, m.p. >300 °C; IR (KBr): *v* 3343, 3031, 2894, 1666, 1640, 1610, 1523, 1506, 1484, 1363, 1245,

1221, 1043, 724, 548 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.94 (s, 1H), 8.01 (d, 1H, J = 7.2 Hz, ArH), 7.88 (d, 1H, J = 7.2 Hz, ArH), 7.79 (t, 1H, J = 7.6 Hz, ArH), 7.73 (t, 1H, J = 7.6 Hz, ArH), 7.29-7.25 (m, 2H, ArH), 7.08 (s, 1H, ArH), 7.01 (t, 21H, J = 8.8 Hz, ArH), 6.81 (s, 1H, ArH), 5.93 (d, 2H, J = 21.4 Hz, OCH_2O), 5.38 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.9, 166.9, 147.1, 144.7, 140.1, 135.3, 133.1, 133.0, 130.8, 130.0, 129.5, 129.4, 125.9, 117.6, 115.6, 115.4, 112.1, 109.1, 101.7, 98.9, 37.7; Anal. Calc. for $C_{24}H_{14}FNO_4$: C 72.18, H 3.53, N 3.51; found: C 72.35, H 3.49, N 3.55.

12-(2-Fluorophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4h**): deongaree power, m.p. 275-276 °C; IR (KBr): v 3341, 3079, 2904, 1667, 1640, 1613, 1588, 1527, 1504, 1484, 1360, 1240, 1039, 724 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.95 (s, 1H), 8.02 (d, 1H, J = 7.6 Hz, ArH), 7.85-7.70 (m, 3H, ArH), 7.28 (t, 1H, J = 7.2 Hz, ArH), 7.16-7.00 (m, 4H, ArH), 6.61 (s, 1H, ArH), 5.91 (d, 2H, J = 26.0 Hz, OCH_2O), 5.62 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.9, 166.9, 147.1, 144.7, 140.1, 135.3, 133.1, 133.0, 130.8, 130.0, 129.5, 129.4, 125.9, 117.6, 115.6, 115.4, 112.1, 109.1, 101.7, 98.9, 37.7; Anal. Calc. for $C_{24}H_{14}FNO_4$: C 72.18, H 3.53, N 3.51; found: C 72.11, H 3.60, N 3.48.

12-(4-Nitrophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4i**): deongaree power, m.p. >300 °C; IR (KBr): v 3323, 3070, 2902, 1677, 1637, 1606, 1519, 1481, 1347, 1247, 1037, 726, 533 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.03 (s, 1H), 8.08-8.02 (m, 3H, ArH), 7.87 (d, 1H, J = 7.2 Hz, ArH), 7.81-7.71 (m, 2H, ArH), 7.55 (d, 2H, J = 8.0 Hz, ArH), 7.10 (s, 1H, ArH), 6.83 (s, 1H, ArH), 5.93 (d, 2H, J = 25.6 Hz, OCH_2O), 5.55 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 180.7, 154.9, 147.4, 146.4, 144.8, 140.5, 135.3, 133.1, 133.0, 130.9, 130.0, 129.0, 126.3, 125.9, 124.2, 116.4, 111.0, 109.2, 101.8, 99.1, 37.7; Anal. Calc. for $C_{24}H_{14}N_2O_4$: C 67.61, H 3.31, N 6.57; found: C 67.80, H 3.36, N 6.49.

12-(3-Nitrophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4j**): blue power, m.p. >300 °C; IR (KBr): *v* 3340., 3064, 2886, 1677, 1637, 1606, 1531, 1505, 1480, 1350, 1247, 1237, 1035, 736, 535 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.05 (s, 1H), 8.13 (s, 1H), 8.03-7.70 (m, 6H, ArH), 7.49 (t, 1H, *J* = 8.0 Hz, ArH), 7.11 (s, 1H, ArH), 6.87 (s, 1H, ArH), 5.93 (d, 2H, *J* = 22.0 Hz, OCH_2O), 5.58 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 180.7, 149.8, 148.3, 147.3, 144.9, 140.4, 135.3, 134.7, 133.1, 133.0, 130.9, 130.4, 130.1, 126.3, 125.9, 122.3, 121.8, 116.7, 111.2, 109.3, 101.8, 99.0, 37.4; Anal. Calc. for $C_{24}H_{14}N_2O_4$: C 67.61, H 3.31, N 6.57; found: C 67.69, H 3.28, N 6.51.

12-(3,4-dichlorophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4k**): deongaree power, m.p. >300 °C; IR (KBr): *v* 3333, 2962, 2922, 2853, 1665, 1637, 1609, 1523, 1504, 1482, 1362, 1243, 1094, 1040, 801, 724 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.99 (s, 1H), 8.02 ((d, 1H, *J* = 6.8 Hz, ArH), 7.90-7.71 (m, 3H, ArH), 7.46 (s, 1H, ArH), 7.45 (d, 1H, *J* = 8.4 Hz, ArH), 7.23 (d, 1H, *J* = 8.4 Hz, ArH), 7.09 (s, 1H), 6.86 (s, 1H, ArH), 5.93 (d, 2H, *J* = 22.0 Hz, OCH_2O), 5.41 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.7, 148.8, 147.3, 144.8, 140.4, 135.2, 133.0, 131.3, 131.1, 130.9, 130.1, 130.0, 129.6, 129.3, 128.2, 126.3, 125.9, 116.7, 111.0, 109.2, 101.8, 99.0, 37.5; Anal. Calc. for $C_{24}H_{13}Cl_2NO_4$: C 64.02, H 2.91, N 3.11; found: C 64.12, H 2.86, N 3.19.

12-(2,4-dichlorophenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4l**): blue power, m.p. >300 °C; IR (KBr): *v* 3346, 2896, 1668, 1639, 1608, 1523, 1504, 1479, 1360, 1242, 1222, 1040, 725 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.96 (s, 1H), 8.02 ((d, 1H, *J* = 7.2 Hz, ArH), 7.83-7.72 (m, 3H, ArH), 7.51 (s, 1H, ArH), 7.33 (d, 1H, *J* = 8.4 Hz, ArH), 7.22 (d, 1H, *J* = 8.0 Hz, ArH), 7.05 (s, 1H), 6.58 (s, 1H, ArH), 5.92 (d, 2H, *J* = 25.6 Hz, OCH_2O), 5.81 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.7, 180.6, 147.3, 145.0, 144.7, 140.7, 136.9, 135.3, 133.0, 132.4, 131.9, 131.7, 130.8, 129.6, 128.9, 128.5, 126.3, 125.8, 116.7, 111.2, 107.9, 101.8, 99.2, 37.7; Anal. Calc. for

$C_{24}H_{13}Cl_2NO_4$: C 64.02, H 2.91, N 3.11; found: C 64.06, H 2.98, N 3.05.

12-(2,5-dimethoxyphenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4m**): deongaree power, m.p. 231-232 ^{o}C; IR (KBr): *v* 3351, 2905, 2833, 1666, 1637, 1607, 1522, 1499, 1486, 1365, 1239, 1218, 1044, 721 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.83 (s, 1H), 8.03 (d, 1H, J = 7.6 Hz, ArH), 7.85-7.71 (m, 4H, ArH), 7.01 (s, 1H, ArH), 6.87 (s, 1H), 6.75-6.62 (m, 2H, ArH), 5.908 (d, 2H, J = 28.4 Hz, OCH_2O), 5.68 (s, 1H, CH), 3.81 (s, 3H, OCH_3), 3.28 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 180.9, 180.7, 153.9, 150.0, 146.8, 144.4, 141.0, 138.1, 135.2, 133.2, 132.9, 130.8, 129.8, 125.8, 120.8, 118.3, 115.7, 113.2, 111.8, 111.4, 108.4, 101.5, 98.8, 56.9, 55.6, 34.6; Anal. Calc. for $C_{26}H_{19}NO_6$: C 70.74, H 4.34, N 3.17; found: C 70.82, H 4.29, N 3.23.

12-(3-Bromo-4-methoxyphenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4n**): deongaree power, m.p. 280-281 ^{o}C; IR (KBr): *v* 3340, 3065, 2901, 1675, 1636, 1602, 1569, 1502, 1478, 1365, 1243, 1037, 727 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.92 (s, 1H), 8.01 (d, 1H, J = 7.6 Hz, ArH), 7.88 (d, 1H, J = 7.6 Hz, ArH), 7.81-7.70 (m, 2H, ArH), 7.44 (d, 1H, J = 1.6 Hz, ArH), 7.18-7.16 (m, 1H, ArH), 7.07 (s, 1H, ArH), 6.92 (d, 1H, J = 8.8 Hz, ArH), 6.82 (s, 1H), 5.92 (d, 2H, J = 20.8 Hz, OCH_2O), 5.32 (s, 1H, CH), 3.72 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.8, 154.3, 147.1, 144.7, 141.9, 140.0, 135.3, 133.1, 133.0, 131.8, 130.8, 130.0, 128.2, 125.9, 117.5, 115.4, 113.2, 111.9, 110.9, 109.2, 101.7, 98.9, 56.7, 37.8; Anal. Calc. for $C_{25}H_{16}BrNO_5$: C 61.24, H 3.29, N 2.86; found: C 61.30, H 3.20, N 2.91.

12-(3,4,5-trimethoxyphenyl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4o**): blue power, m.p. >300 ^{o}C; IR (KBr): *v* 3344, 2908, 2835, 1664, 1637, 1605, 1590, 1519, 1488, 1424, 1360, 1240, 1126, 1032, 722 cm^{-1}; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 9.86 (s, 1H), 8.02 (d, 1H, J = 7.2 Hz, ArH), 7.90 (d, 1H, J = 7.6 Hz, ArH), 7.80 (t, 1H, J = 7.2 Hz, ArH), 7.73 (t, 1H, J = 7.2 Hz, ArH), 7.06 (s, 1H, ArH), 6.88 (s, 1H), 6.54 (s, 2H, ArH), 5.92 (d, 2H, J = 20.4 Hz, OCH_2O), 5.28 (s, 1H, CH),

3.66 (s, 6H, $2OCH_3$), 3.55 (s, 3H, OCH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.1, 180.9, 153.3, 147.0, 144.6, 143.9, 140.1, 136.6, 135.2, 133.1, 132.9, 130.9, 129.9, 126.2, 125.9, 117.8, 112.1, 109.2, 105.2, 101.6, 98.8, 60.3, 56.4, 41.0; Anal. Calc. for $C_{27}H_{21}NO_7$: C 68.78, H 4.49, N 2.97; found: C 68.69, H 4.53, N 3.05.

12-(Furan-2-yl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4p**): blue power, m.p. >300 ^oC; IR (KBr): v 3336, 3067, 2900, 1672, 1637, 1608, 1572, 1507, 1485, 1364, 1240, 1039, 728, 522 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.98 (s, 1H), 8.03 (d, 1H, J = 7.2 Hz, ArH), 7.94 (d, 1H, J = 7.6 Hz, ArH), 7.82 (t, 1H, J = 7.6 Hz, ArH), 7.75 (t, 1H, J = 7.6 Hz, ArH), 7.39 (s, 1H, ArH), 7.05 (s, 1H, ArH), 6.90 (s, 1H), 6.23-6.03 (m, 2H, ArH), 5.95 (d, 2H, J = 15.6 Hz, OCH_2O), 5.46 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.8, 157.9, 147.3, 144.6, 142.2, 140.6, 135.3, 133.1, 130.8, 130.4, 129.2, 126.3, 125.9, 115.0, 110.9, 109.2, 108.8, 105.4, 101.7, 98.9, 34.5; Anal. Calc. for $C_{22}H_{13}NO_5$: C 71.15, H 3.53, N 3.77; found: C 71.02, H 3.56, N 3.81.

12-(Thiophen-2-yl)-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4q**): deongaree power, m.p. 214-215 ^oC; IR (KBr): v 3328, 3065, 2890, 1671, 1605, 1499, 1481, 1357, 1240, 1036, 728 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 10.06 (s, 1H), 8.02 (d, 1H, J = 7.6 Hz, ArH), 7.95 (d, 1H, J = 7.6 Hz, ArH), 7.84-7.72 (m, 2H, ArH), 7.21-7.19 (m, 1H, ArH), 7.08 (s, 1H, ArH), 6.98-6.76 (m, 2H, ArH), 6.03 (s, 1H), 5.96 (d, 2H, J = 15.6 Hz, OCH_2O), 5.66 (s, 1H, CH); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.0, 180.8, 157.9, 147.3, 144.6, 142.2, 140.6, 135.3, 133.1, 130.8, 130.4, 129.2, 126.3, 125.9, 115.0, 110.9, 109.2, 108.8, 105.4, 101.7, 98.9, 34.5; Anal. Calc. for $C_{22}H_{13}NO_4S$: C 68.21, H 3.38, N 3.62; found: C 68.25, H 3.29, N 3.66.

12-Methyl-5,10-dihydro-benzo[i][1,3]dioxolo[4,5-b]acridine-6,11-dione (**4r**): blue power, m.p. >300; IR (KBr): v 3307, 3116, 3066, 2958, 2920, 1681, 1638, 1606, 1571, 1514, 1478, 1372, 1240, 1045, 722 cm^{-1}; ^1H NMR (400 MHz, DMSO-d_6) δ: 9.70 (s, 1H), 7.98 (t, 1H, J = 7.2 Hz, ArH),

7.82 (t, 1H, J = 6.8 Hz, ArH), 7.73 (t, 1H, J = 7.2 Hz, ArH), 6.99 (s, 1H, ArH), 6.87 (s, 1H), 5.96 (d, 2H, J = 6.4 Hz, OCH_2O), 4.23-4.19 (m, 1H, CH), 1.15 (d, 3H, J = 6.8 Hz, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 181.1, 180.8, 146.7, 144.6, 140.1, 135.2, 133.3, 132.9, 130.7, 130.2, 126.2, 125.8, 119.4, 113.7, 108.6, 101.5, 98.6, 30.0, 25.7; Anal. Calc. for $C_{19}H_{13}NO_4$: C 71.47, H 4.10, N 4.39; found: C 71.40, H 4.03, N 4.42.

Cytotoxicity assay

Cell viability for all cell lines was determined using the 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) colorimetric assay. Compounds **4a-4r** were subjected to cytotoxic evaluation against Hela and HepG2 cell lines employing the colorimetric method. 4-Aza-podophyllotoxin was used as the reference substance.

MTT was dissolved in saline to make the concentration of 5 mg/μL as a stock solution. Cancer cells (3×10^3 cells) suspended in 100 mg/well of MEM medium containing 10% fetal calf serum were seeded onto a 96-well culture plate. After 24 h pre-incubation at 37 ^oC in a humidified atmosphere of 5% CO_2/95% air to allow cells attachment, various concentrations of test solution (10 μL/well) as listed in Table 3 were added and then incubated for 48 h under the above condition. At the end of the incubation, 10 μL of tetrazolium reagent was added into each well and then incubated at 37 ^oC for 4 h. The supernatant was decanted, and DMSO (100 μL/well) was added to allow formosan solubilization. The optical density (OD) of each well was detected by a microplate reader at 550 nm and for correction at 595 nm. Each determination represents the average means of six replicates. The 50% inhibition concentration (IC_{50}) was determined by curve fitting.

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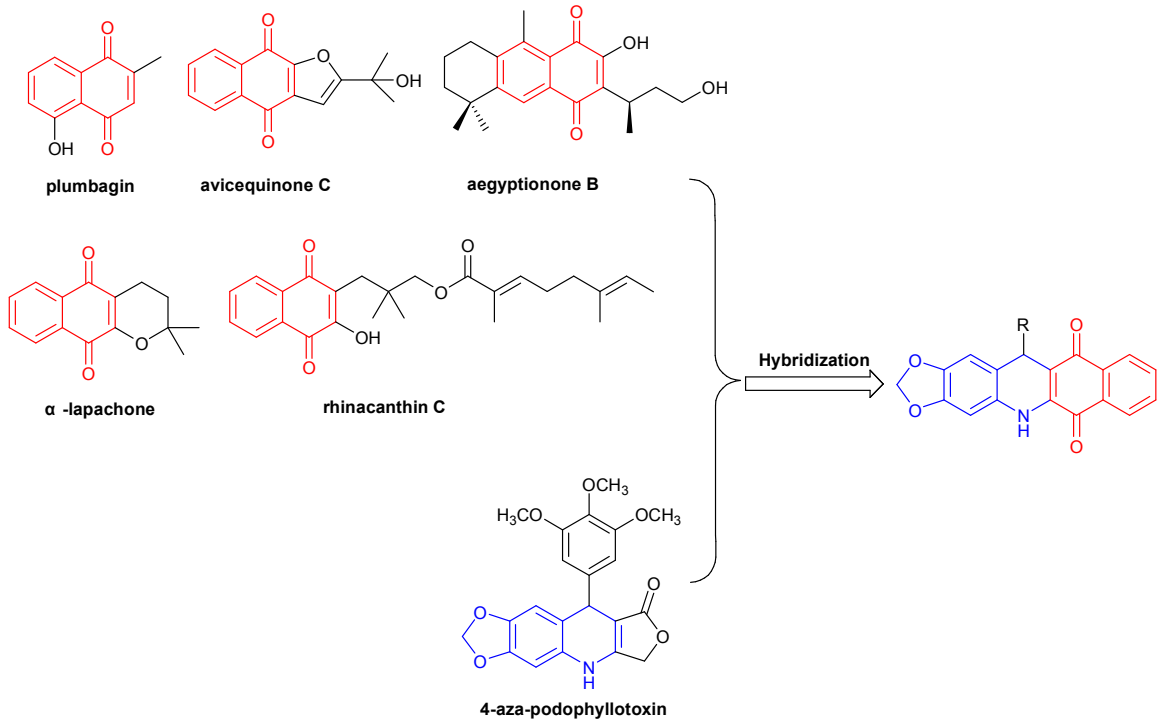
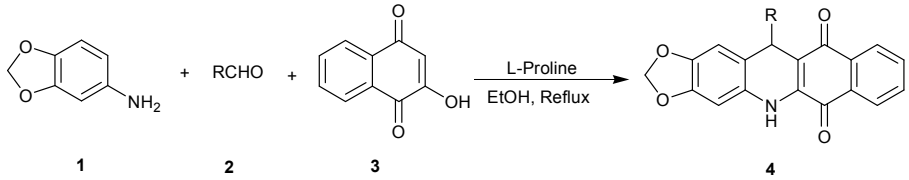


Figure 1. Design of *para*-naphthoquinone-4-aza-podophyllotoxin hybrids



Scheme 1

Table 1 Optimization of the reaction conditions for three-component synthesis of *para*-naphthoquinone-4-aza-podophyllotoxin hybrids[a]

Entry	Catalyst (mol%)	Time (h)	Yield (%)[b]
1	No catalyst	3	46
2	*p*-TsOH (10)	2	63
3	H_2SO_4 (10)	2	52
4	silica sulfuric acid (10)	2	59
5	$ZnCl_2$ (10)	5	55
6	$InCl_3$ (10)	2	69
7	Et_3N (10)	2	70
8	Pyridine (10)	2	68
9	Glycine (10)	2	58
10	L-Leucine (10)	2	67
11	L-Proline (10)	1	93
12	L-Proline (5)	2	78
13	L-Proline (15)	1	92
14	L-Proline (20)	1	93

[a] Reaction conditions: 3,4-methylenedioxyaniline (1 mmol), benzaldehyde (1 mmol), 2-hydroxy-1,4-naphthoquinone (1 mmol); EtOH; reflux.

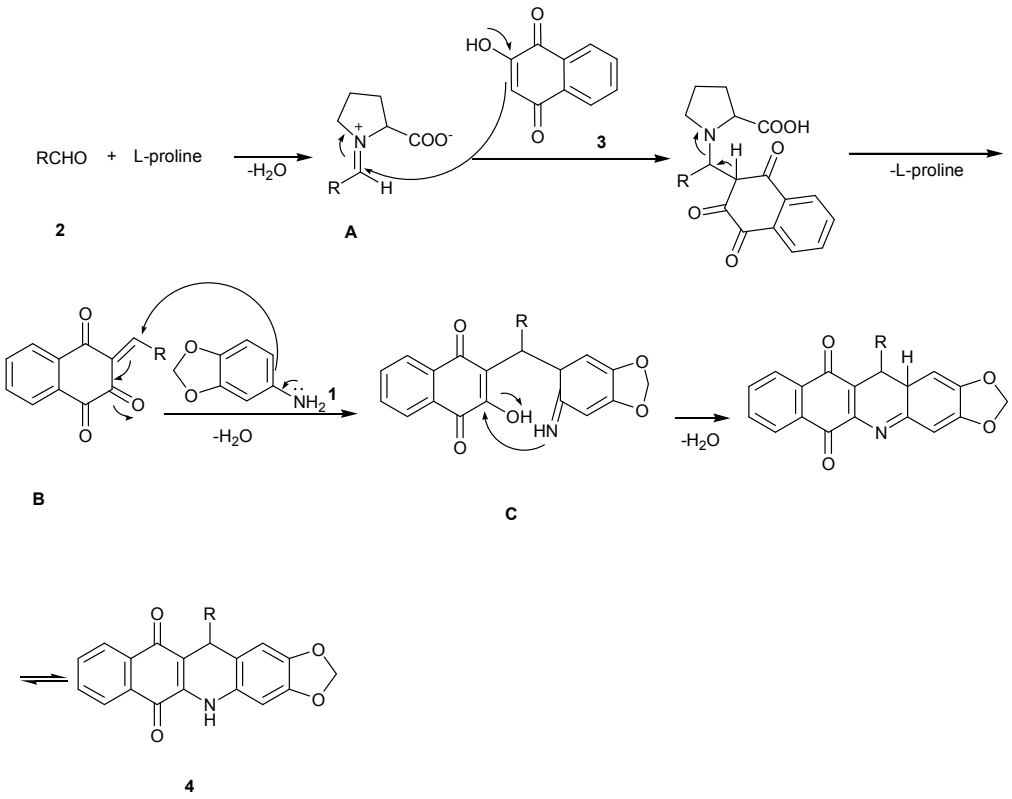
[b] Isolated yield.

Table 2 Preparation of *para*-naphthoquinone-4-aza-podophyllotoxin hybrids[a]

Entry	R	Time(h)	Product	Yield (%)[b]
1	C_6H_5	1	**4a**	93
2	4-Me-C_6H_4	1	**4b**	99
3	4-MeO-C_6H_4	1	**4c**	89
4	3-MeO-C_6H_4	1	**4d**	85
5	4-Cl-C_6H_4	1	**4e**	94
6	2-Cl-C_6H_4	1.5	**4f**	88
7	4-F-C_6H_4	1	**4g**	89
8	2-F-C_6H_4	1.5	**4h**	86
9	4-NO_2-C_6H_4	1	**4i**	94
10	3-NO_2-C_6H_4	1.5	**4j**	89
11	3,4-$(Cl)_2$-C_6H_4	1.5	**4k**	84
12	2,4-$(Cl)_2$-C_6H_4	1.5	**4l**	85
13	2,5-$(MeO)_2$-C_6H_3	1.5	**4m**	82
14	3,5-$(MeO)_2$-C_6H_3	1.5	**4n**	86
15	3,4,5-$(MeO)_3$-C_6H_2	1.5	**4o**	84
16	2-Furanyl	1	**4p**	88
17	2-Thiophenyl	1	**4q**	90
18	Methyl	2	**4r**	72

[a] Reaction conditions: 3,4-methylenedioxyaniline (1 mmol), aldehyde (1 mmol), 2-hydroxy-1,4-naphthoquinone (1 mmol); L-proline (10); EtOH; reflux.

[b] Isolated yield.



Scheme 2

Table 3 Antitumor activities of *para*-naphthoquinone- 4-aza-podophyllotoxin hybrids.

Compound	IC_{50} (μM)[a]			Tumor specificity ratio
	HepG2	Hela	L02	L02/ HepG2
4a	32.83 ± 5.80	25.05 ± 5.51	60.12 ± 7.40	1.83
4b	103.33 ± 21.84	106.86 ± 11.43	>200	>1.93
4c	28.58 ± 9.13	21.61 ± 5.94	51.34 ± 6.73	1.80
4d	>200	>200	>200	-
4e	100.41 ± 14.96	112.34 ± 8.54	>200	>2.00
4f	53.06 ±7.02	45.25 ± 6.77	100.25 ±8.58	1.89
4g	90.37 ± 6.30	99.03 ± 9.66	183.43 ± 8.48	2.03
4h	35.70 ± 4.30	44.96 ± 4.05	80.67 ± 7.48	2.26
4i	>200	>200	>200	-
4j	46.02 ± 3.45	35.20 ± 7.14	91.57 ± 4.68	1.99
4k	>200	>200	>200	-
4l	>200	>200	>200	-
4m	77.48 ± 19.98	77.48 ± 11.84	152.46 ± 18.54	1.97
4n	23.75 ± 4.37	26.75 ± 7.76	54.33 ± 5.25	2.29
4o	14.56 ± 4.37	26.75 ± 4.05	27.44 ± 3.57	1.88
4p	>200	>200	>200	-
4q	>200	>200	>200	-
4r	59.82 ± 6.57	39.58 ± 6.72	105.18 ± 9.96	1.76
4-AP	28.78 ± 2.95	36.11 ± 5.98	42.22 ± 3.32	1.47

[a] The means of triplicates ± SD