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Original article

Studies on novel 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxins as potential anticancer agents☆

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Abstract

A series of 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin congeners have been designed and synthesized with significant regioselectivity by employing Cu(I) catalyzed 1,3-dipolar cycloaddition reaction of *C*4β-azido podophyllotoxin and *C*4β-azido-4′-*O*-demethyl podophyllotoxin with *N*-prop-2-yn-1-ylanilines. These compounds were evaluated for anticancer activity against a panel of seven human cancer cell lines. It was interesting to note that all the compounds exhibited promising activity especially against SF-295 (CNS), HCT-15 (colon) and 502713 (colon) cell lines. Compound **11e** was found to be the most promising in this study.

Keywords: Podophyllotoxin; Regioselectivity; *N*-prop-2-yn-1-ylanilines; Cancer cell lines

1. Introduction

Podophyllum peltatum L. (and *Podophyllum emodi* L., found in China and India) also known as American mandrake or May apple is the rich source of podophyllotoxin. Podophyllotoxin has been known to inhibit the tubulin assembly into microtubules through tubulin binding, but its high toxicity has limited its application as a drug in cancer chemotherapy [1]. Several podophyllotoxin derivatives that are potent inhibitors of mitosis have been synthesized and examined as antitumor agents. Etoposide, **2** [2] and teniposide, **3** [3], the two semi-synthetic epipodophyllotoxin derivatives are in clinical use as antineoplastic agents. Their clinical efficacy is due to their ability to inhibit the enzyme DNA-topoisomerase II. These podophyllotoxin lignans block the catalytic activity of DNA-topoisomerase II by stabilizing a cleavable enzyme–DNA complex in which the DNA is cleaved and covalently linked to the enzyme [4]. Both etoposide and teniposide are clinically useful against various cancers, including small-cell lung cancer, testicular carcinoma, lymphoma and Kaposi's sarcoma [1,5]; however, their therapeutic uses are often hindered by acquired drug-resistance. In another podophyllotoxin glycoside derivative, NK611, **4**, the 2″-hydroxyl group in the glucose ring of **2** has been replaced with an *N*,*N*-dimethylamino moiety. It is currently undergoing phase I clinical evaluation [6]. In order to obtain better therapeutic agents, extensive synthetic efforts have been devoted to overcome drug-resistance and improve topoisomerase II inhibition [7,8].

The recent synthetic studies on podophyllotoxin have been focused on the synthesis of the *C*-4 non-sugar substituted analogs, which exhibit improved topoisomerase II inhibition. The replacement of the *C*-4 sugar moiety of etoposide and teniposide with a non-sugar substituent has improved the therapeutic value of etoposide [9]. The *C*-4 non-sugar substituent can be linked through O-, S- or N-linkage. In general, the O-linked (ethers, esters) and S-linked (thioethers) compounds are less active in comparison to the N-linked congeners [3,10–12] like GL-331 and NPF (Fig. 1).

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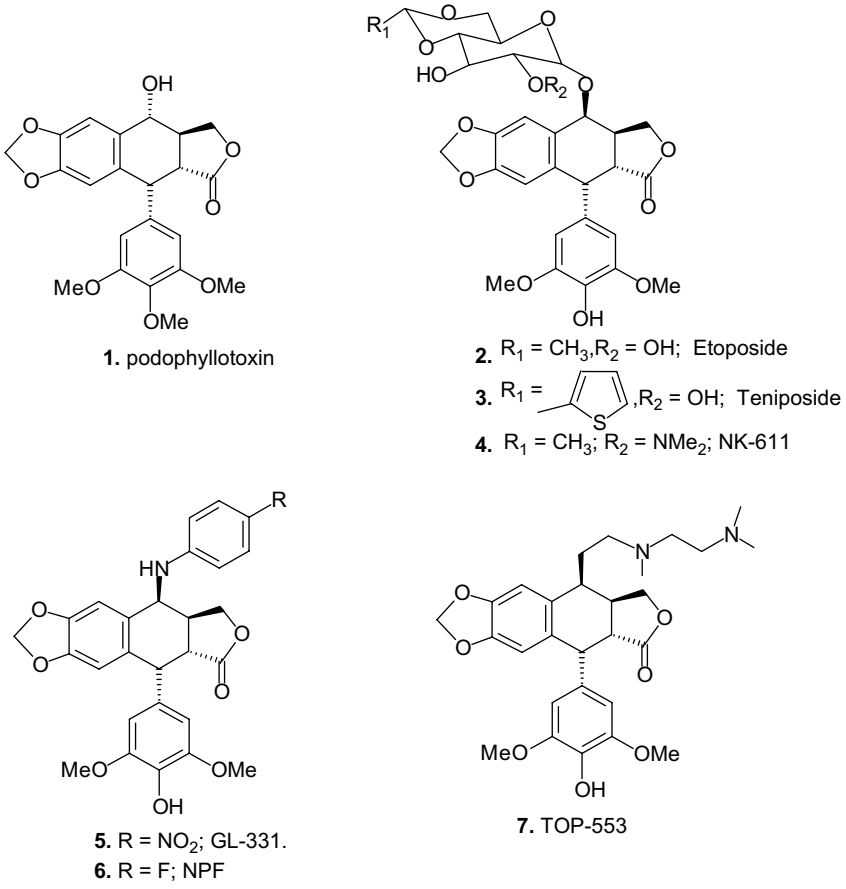


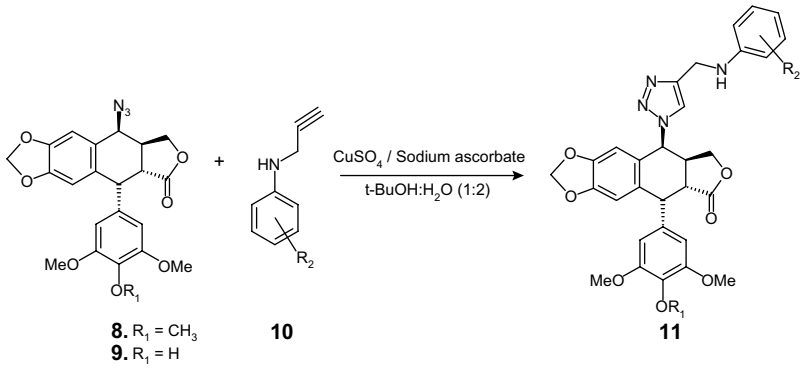
Fig. 1. Structure of some podophyllotoxin derivatives.

GL-331 and TOP-53 [13] have been found to have potential activity, especially selective in resistant malignancies [14] and nonsmall-cell lung cancers [15], respectively. Both of them showed topoisomerase II inhibitory activity, antitumor spectra, and drug-resistance profiles significantly different from those of **2**, which suggested an important role of various C-4 substitutions on the activity profiles of **2**-related analogs and the feasibility of optimizing this compound class through rational C-4 modification. This postulation coincides with the composite pharmacophore model proposed by MacDonald et al. [4] and the comparative molecular field analysis (CoMFA) model generated by Cho et al. [16]. Both models indicated that the C-4 molecular area could accommodate considerable structural diversity. Wang and co-workers have reported a brief synthesis of 4β-[(5-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives as a new class of antitumor compounds [17]. This prompted us to synthesize a library of 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives as new anticancer compounds using click-chemistry protocols. Click chemistry enables a modular approach to generate these novel pharmacophores utilizing a collection of reliable chemical reactions [18]. Of particular interest is the Huisgen [3 + 2] cycloaddition between a terminal alkyne and an azide to generate substituted 1,2,3-triazoles [19]. Using this click reaction a series of novel regioselective 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives have been synthesized and screened for anticancer activity against a panel of seven human cancer cell lines. From the IC_{50} values it is found that all the compounds are having promising anticancer activity, better than the standard compounds like podophyllotoxin and etoposide.

2. Results and discussion

2.1. Chemistry

As illustrated in Scheme 1, 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives were synthesized by the cycloaddition reaction of *C*4β-azido podophyllotoxin, **8** and *C*4β-azido-4′-*O*-demethyl podophyllotoxin, **9** with *N*-prop-2-yn-1-ylanilines, obtained by the reaction of anilines with propargyl bromide in the presence of potassium carbonate in acetone under reflux conditions. *C*4β-Azido-4′-*O*-demethyl podophyllotoxin was synthesized according to the literature procedure in which podophyllotoxin was first converted to 4′-*O*-demethyl epipodophyllotoxin by the inversion of 4-hydroxyl and simultaneous demethylation of 4′-OCH_3 using CH_3SO_3H and NaI in dichloromethane to the corresponding iodo-derivative followed by hydrolysis with H_2O/Me_2CO/barium carbonate to 4′-*O*-demethyl epipodophyllotoxin [20]. The key intermediate, 4β-azido-4′-*O*-demethyl podophyllotoxin was obtained in excellent yield by treating the latter with TFA and NaN_3. On the other hand, *C*4β-azido-podophyllotoxin was obtained by the reaction of podophyllotoxin with



Scheme 1. Click-chemistry strategy for the synthesis of novel 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives.

CH_3SO_3H and NaI in acetonitrile followed by hydrolysis with H_2O/Me_2CO/barium carbonate and reaction with NaN_3 in TFA. The azides **8** and **9** obtained were allowed to react with the above terminal alkynes, in the presence of $CuSO_4 \cdot 5H_2O$ and sodium ascorbate, in *t*-butyl alcohol and water (1:2) at room temperature to yield selectively 4β-[(4-substituted)-1,2,3-triazol-1-yl] derivatives in excellent yields (84–90%). Using this click-chemistry protocol a series of 10 compounds differing in substitution at R_1 and R_2 have been synthesized (Table 1).

All the products were characterized by ^{1}H NMR, ^{13}C NMR, IR, ESI-MS and elemental analyses, which were found to be within the range of ±0.4 from theoretical values. In ^{1}H NMR, cyclization of azides to triazoles was confirmed by resonance of H-5 of triazole ring in aromatic region and that of *C*4α-H around δ 6.

2.2. Biology

All the compounds were assayed for *in vitro* cytotoxicity against a panel of seven human cancer cell lines including DU-145, PC-3 (prostrate), SF-295 (CNS), HCT-15, 502713 (Colon), HEP-2 (liver) and A-549 (lung) using sulforhodamine B [21,22]. Podophyllotoxin and etoposide were taken as reference compounds and the results are reported in terms of IC_{50} values (Table 2).

From the IC_{50} values, it is clear that all the compounds have significant cytotoxic activity against prostrate, CNS, colon and liver derived cancer cell lines. However, it may be noted that these compounds showed comparatively lesser activity against A-549, a lung derived cancer cell line. Compound **11e**, with *m*-nitro-substitution on aniline part with trimethoxy moiety on ring E of lignan, showed significant cytotoxicity against DU-145, PC-3, SF-295, HCT-15 and 502713 cell lines, however, its activity was found to be maximum against HCT-15 cell line with IC_{50} value 0.04 μM. Compounds **11a–11c**, **11g** and **11h** were also found to have promising activity against the above mentioned cell lines. From the IC_{50} values, it is clear that the compounds with trimethoxy moiety in ring E are having more cytotoxic activity than those with dimethoxy moiety.

In conclusion, a series of 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives were synthesized and screened for anticancer activity against a panel of seven human cancer cell lines. From the data it was found that all the compounds are having promising anticancer activity and compound **11e** was found to be the most active in this study.

3. Experimental

Melting points were recorded on Buchi Melting point apparatus D-545 and IR spectra (KBr) on Bruker Vector 22 instrument. NMR spectra were recorded on Bruker DPX200 instrument in $CDCl_3$ with TMS as an internal standard. Chemical shift values are reported in δ (ppm) and coupling constants in hertz. Mass spectra were recorded on ESI-esquire 3000 Bruker Daltonics instrument. The progress of all reactions was monitored by TLC on 2 × 5 cm pre-coated silica gel 60 F_{254} plates of thickness 0.25 mm (Merck). The chromatograms were visualized under UV 254–366 nm and iodine.

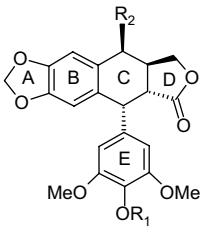
3.1. Click chemistry—general procedure

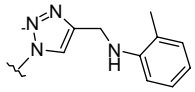
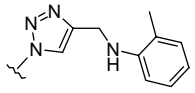
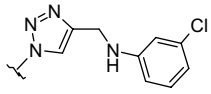
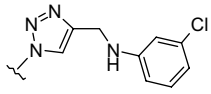
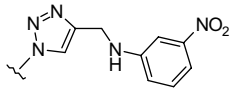
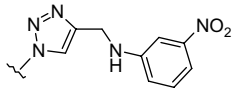
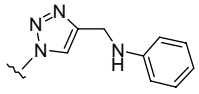
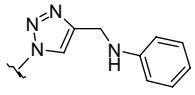
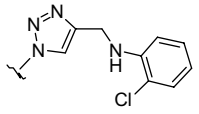
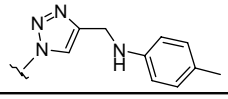
To a solution of **9** (1 mmol) in *t*-butyl alcohol and water (1:2, 8 mL) was added $CuSO_4 \cdot 5H_2O$ (1 mmol), sodium ascorbate (2 mmol) followed by 4β-azido-podophyllotoxin (44 mg, 0.1 mmol). The reaction mixture was stirred at room temperature for 8 h. After completion, the reaction mixture was diluted with 80 mL of water and extracted with ethylacetate (2 × 20 mL). The combined extracts were washed with brine, dried over Na_2SO_4 and evaporated *in vacuo*. The crude product obtained was precipitated in diethyl ether to yield the pure product **11**.

3.1.1. 9-[4-(o-Tolylamino-methyl)-[1,2,3]-triazol-1-yl]-5-(3,4,5-trimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11a**)

White solid; mp: 128–130 °C; $[\alpha]_D^{25}$ −41.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3H), 3.06 (m, 1H), 3.23 (m, 2H), 3.64 (s, 6H), 3.89 (s, 3H), 4.03 (d, 1H, $J = 2.29$ Hz), 4.40–4.51 (m, 3H), 4.75 (d, 1H, $J = 4.76$ Hz), 6.08 (m, 3H), 6.33 (s, 2H), 6.59–6.76 (m, 4H), 7.13 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 17.4, 33.7, 37.1, 41.6, 43.5, 56.3, 60.7, 67.4, 71.4, 101.9, 108.3,

Table 1
Various podophyllotoxin 4β-[(4-substituted)-1,2,3-triazol-1-yl] derivatives



Entry	R_1	R_2	Yield[a]
11a	CH_3		88
11b	H		86
11c	CH_3		87
11d	H		88
11e	CH_3		84
11f	H		88
11g	CH_3		87
11h	H		83
11i	CH_3		88
11j	CH_3		90

[a] Isolated yields.

108.8, 110.5, 118.2, 124.7, 127.1, 130.2, 133.3, 134.3, 137.7, 148.09, 149.45, 152.8, 173.1; IR (KBr): 3425, 1778.6, 1588.5, 1506.3, 1237.8, 1126.4, 751.0 cm^{-1}; ESI-MS: 607 (M + Na).

*3.1.2. 5-(4-Hydroxy-3,5-dimethoxy-phenyl)-9-[4-(o-tolylamino-methyl)-[1,2,3]triazol-1-yl]-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11b**)*

White solid; mp: 145–148 °C; $[\alpha]_D^{25}$ −25.3 (*c* 0.47, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 2.16 (s, 3H), 3.02 (m, 1H), 3.18 (m, 2H), 3.79 (s, 6H), 3.99 (d, 1H, *J* = 2.28 Hz), 4.35 (m, 1H), 4.47 (s, 2H), 4.71 (d, 1H, *J* = 4.82 Hz), 6.02 (m, 3H), 6.32 (s, 2H), 6.59–6.73 (m, 4H), 7.11 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 18.7, 33.7, 37.6, 40.1, 42.9, 56.99, 60.3, 67.8, 103.5, 108.4, 109.5, 112.2, 117.1, 122.1, 127.2, 129.1, 130.4, 134.7, 135.09, 144.8, 145.4, 146.8, 172.4; IR (KBr): 3418.1, 1778.2, 1605.1, 1485.07, 1231.9, 1115.4, 762.8 cm^{-1}; ESI-MS: 593.1 (M + Na).

Table 2
IC_{50} values (μM) of 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin against a panel of human cancer cell lines

Entry	Prostate		CNS	Colon		Liver	Lung
	DU-145	PC-3	SF-295	HCT-15	502713	HEP-2	A-549
11a	1.06	1.09	1.94	0.36	0.78	9.14	7.88
11b	1.19	1.53	0.96	0.14	0.62	1.50	5.17
11c	0.93	1.18	0.84	0.34	0.71	1.37	4.99
11d	4.16	1.37	3.11	4.22	0.98	1.22	8.63
11e	0.65	0.74	0.68	0.04	0.56	3.65	5.78
11f	4.37	6.63	4.20	1.55	1.19	4.10	7.43
11g	1.12	0.88	0.78	0.14	0.61	4.64	6.10
11h	1.09	1.03	0.70	0.40	0.68	2.96	7.46
11i	3.57	3.45	1.00	1.43	1.88	1.26	9.24
11j	2.75	4.49	6.03	1.69	1.34	3.27	7.41
1	2.97	18.9	5.69	1.00	3.88	1.42	7.63
2	3.85	17.4	13.3	1.48	2.42	2.15	5.62

*3.1.3. 9-{4-[(3-Chloro-phenylamino)-methyl]-[1,2,3]triazol-1-yl}-5-(3,4,5-trimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11c**)*

White solid; mp: 136–138 °C; $[\alpha]_D^{25}$ −24.1 (*c* 0.47, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 3.01 (m, 1H), 3.21 (m, 2H), 3.76 (s, 6H), 3.81 (s, 3H), 4.12–4.21 (m, 1H), 4.40–4.53 (m, 3H), 4.73 (d, 1H, *J* = 4.84 Hz), 5.99–6.07 (m, 3H), 6.51 (s, 2H), 6.51–6.72 (m, 5H), 7.07 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 37.1, 39.7, 41.6, 43.6, 56.3, 58.7, 60.7, 67.3, 102.0, 108.3, 108.7, 110.5, 111.7, 113.0, 113.2, 118.2, 118.5, 124.5, 130.2, 133.2, 134.2, 135.0, 137.7, 148.0, 148.5, 149.5, 152.8, 173.1; IR (KBr): 3389.2, 1778.6, 1598.5, 1505.3, 1485.2, 1238.0, 1002.6, 766.7 cm^{-1}; ESI-MS: 627 (M + Na).

*3.1.4. 9-{4-[(3-Chloro-phenylamino)-methyl]-[1,2,3]triazol-1-yl}-5-(4-hydroxy-3,5-dimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]-dioxol-6-one (**11d**)*

White solid; mp: 144–147 °C; $[\alpha]_D^{25}$ −29.5 (*c* 0.47, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 3.18 (m, 1H), 3.22 (m, 2H), 3.79 (s, 6H), 3.92 (d, 1H, *J* = 2.27 Hz), 4.38 (m, 3H), 4.72 (d, 1H, *J* = 4.66 Hz), 5.99–6.05 (m, 3H), 6.32 (s, 2H), 6.47–6.76 (m, 5H), 7.08 (m, 2H); ^{13}C NMR ($CDCl_3$): δ 37.1, 39.7, 41.6, 56.3, 58.7, 67.3, 72.8, 102.0, 108.3, 108.7, 110.5, 111.7, 113.0, 118.2, 124.5, 130.2, 134.2, 135.0, 137.7, 148.0, 149.5, 152.8, 173.1; IR (KBr): 3405.5, 1777.5, 1598.3, 1505.3, 1232.2, 1114.5, 1036.9, 765.9 cm^{-1}; ESI-MS: 590 (M + Na).

*3.1.5. 9-{4-[(3-Nitro-phenylamino)-methyl]-[1,2,3]triazol-1-yl}-5-(3,4,5-trimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11e**)*

White solid; mp: 208–211 °C; $[\alpha]_D^{25}$ −42.2 (*c* 0.40, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.08 (m, 1H), 3.29 (m, 2H), 3.82 (s, 6H), 3.87 (s, 3H), 4.07–4.21 (m, 1H), 4.40–4.52 (m, 3H), 4.80 (d, 1H, *J* = 4.82 Hz), 6.10 (m, 3H), 6.37 (s, 2H), 6.61 (s, 1H), 6.70 (s, 1H), 6.98 (d, 1H, *J* = 8.04 Hz), 7.24–7.45 (m, 3H), 7.62 (d, 1H, *J* = 9.12 Hz); ^{13}C NMR ($CDCl_3$): δ 34.9, 37.7, 40.1, 42.7, 56.4, 58.6, 60.0, 70.3, 105.5, 107.6, 108.3, 108.6, 110.1, 112.1, 120.3, 124.1, 125.2, 130.2, 133.3, 134.1, 137.1, 148.3, 149.5, 152.2, 173.2; IR (KBr): 3389.5, 1778.2, 1588.7, 1530.1, 1348.8, 1237.7, 1125.6, 1092.95, 1002.35, 736.4, 675.3 cm^{-1}; ESI-MS: 638 (M + Na).

*3.1.6. 5-(4-Hydroxy-3,5-dimethoxy-phenyl)-9-{4-[(3-nitro-phenylamino)-methyl]-[1,2,3]triazol-1-yl}-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11f**)*

White solid; mp: 221–223 °C; $[\alpha]_D^{25}$ −44.1 (*c* 0.50, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.08 (m, 1H), 3.29 (m, 2H), 3.82 (s, 6H), 4.07–4.23 (m, 1H), 4.40–4.52 (m, 3H), 4.80 (d, 1H, *J* = 4.82 Hz), 6.10 (m, 3H), 6.37 (s, 2H), 6.59 (s, 1H), 6.68 (s, 1H), 6.98 (d, 1H, *J* = 7.88 Hz), 7.24–7.45 (m, 3H), 7.62 (d, 1H, *J* = 9.12 Hz); ^{13}C NMR ($CDCl_3$): δ 34.9, 37.7, 40.1, 42.7, 56.4, 61.7, 70.3, 105.5, 107.6, 108.3, 108.6, 110.1, 112.1, 120.3, 124.1, 125.2, 130.2, 133.3, 134.1, 137.1, 148.3, 149.5, 152.2, 173.2; IR (KBr): 3389.5, 1778.2, 1588.7, 1530.1, 1348.8, 1237.7, 1125.6, 1092.95, 1002.35, 736.4, 675.3 cm^{-1}; ESI-MS: 624 (M + Na).

*3.1.7. 9-(4-Phenylaminomethyl-[1,2,3]triazol-1-yl)-5-(3,4,5-trimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11g**)*

White solid; mp: 168–171 °C; $[\alpha]_D^{25}$ −33.4 (*c* 0.44, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.99 (m, 1H), 3.16 (m, 2H), 3.76 (s, 6H), 3.94 (s, 3H), 3.94 (d, 1H, *J* = 2.28 Hz), 4.36–4.43 (m, 3H), 4.70 (d, 1H, *J* = 4.67 Hz), 6.00 (m, 3H), 6.31 (s, 2H), 6.58–6.79 (m, 5H), 7.12–7.22 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 38.1, 41.1, 42.3, 47.1, 57.3, 59.6, 61.7, 103.6, 108.9, 110.1, 112.7, 115.8, 119.4, 123.5, 125.4, 132.2, 133.7, 134.9, 145.4, 149.5, 150.4, 157.2, 173.3; IR (KBr): 3405.4, 1777.6, 1602.4, 1504.2, 1485.1, 1237.5, 1125.7, 752.2 cm^{-1}; ESI-MS: 593 (M + Na).

*3.1.8. 5-(4-Hydroxy-3,5-dimethoxy-phenyl)-9-(4-phenylaminomethyl-[1,2,3]triazol-1-yl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11h**)*

White solid; mp: 81–182 °C; $[\alpha]_D^{25}$ −29.9 (*c* 0.61, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.98 (m, 1H), 3.19 (m, 2H), 3.80 (s, 6H), 3.95 (d, 1H, *J* = 2.28 Hz), 4.36–4.44 (m, 3H), 4.72 (d, 1H, *J* = 4.71 Hz), 5.99–6.06 (m, 3H), 6.32 (s, 2H), 6.59–6.85 (m, 5H), 7.12–7.27 (m, 3H); ^{13}C NMR ($CDCl_3$): δ 38.1, 41.1, 42.3, 47.1, 57.3, 59.6, 103.6, 108.9, 110.1, 112.7, 115.8, 119.4, 123.5, 125.4, 132.2, 133.7, 134.9, 145.4, 149.5, 150.4, 157.2, 173.3; IR (KBr): 3405.4, 1777.6, 1602.4, 1504.2, 1485.1, 1237.5, 1125.7, 752.2 cm^{-1}; ESI-MS: 579 (M + Na).

*3.1.9. 9-{4-[(2-Chloro-phenylamino)-methyl]-[1,2,3]triazol-1-yl}-5-(3,4,5-trimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11i**)*

White solid; mp: 167–170 °C; $[\alpha]_D^{25}$ −25.7 (*c* 0.46, $CHCl_3$); ^{1}H NMR (200 MHz, $CDCl_3$): δ 2.96 (m, 1H), 3.20 (m, 2H), 3.77 (s, 6H), 3.82 (s, 3H), 3.93 (d, 1H, *J* = 2.35 Hz), 4.41 (br s, 3H), 4.74 (d, 1H, *J* = 4.71 Hz), 6.01–6.09 (m, 3H), 6.32 (s, 2H), 6.48–6.73 (m, 5H), 7.09 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 36.8, 37.7, 40.4, 42.2, 56.9, 59.3, 61.3, 67.9, 102.5, 105.1, 108.8, 110.5, 111.7, 113.0, 113.2, 118.2, 118.5, 124.5, 130.2, 133.2, 134.2, 135.0, 137.7, 148.0, 148.5, 149.5, 153.8, 173.6; IR (KBr): 3389.2, 1778.6, 1598.5, 1505.3, 1485.2, 1238.0, 1002.6, 766.7 cm^{-1}; ESI-MS: 627 (M + Na).

*3.1.10. 9-[4-(p-Tolylamino-methyl)-[1,2,3]triazol-1-yl]-5-(3,4,5-trimethoxy-phenyl)-5,8,8a,9-tetrahydro-5aH-furo[3′,4′:6,7]naphtho[2,3-d][1,3]dioxol-6-one (**11j**)*

White solid; mp: 146–148 °C; $[\alpha]_D^{25}$ −33.4 (*c* 0.75, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.19 (s, 3H), 3.06 (m, 1H), 3.23 (m, 2H), 3.64 (s, 6H), 3.89 (s, 3H), 4.03 (d, 1H, *J* = 2.29 Hz), 4.40–4.51 (m, 3H), 4.75 (d, 1H, *J* = 4.76 Hz), 6.08 (m, 3H), 6.32 (s, 2H), 6.63 (s, 1H), 6.66 (s, 1H), 6.79 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): 18.1, 34.9, 41.5, 42.8, 56.5, 58.7, 67.3, 101.9, 107, 8, 108.5, 110.4, 114.7, 124.5, 129.6, 130.2, 130.8, 133.63, 134.4, 134.4, 146.6, 147.7, 155.8, 173.1; IR (KBr): 3437, 2905, 1768, 1505.3, 1486, 1238.0, 1095, 1002.6, 766.7 cm^{-1}; ESI-MS: 607 (M + Na).

3.2. Biological assay

The effect of 4β-[(4-substituted)-1,2,3-triazol-1-yl] podophyllotoxin derivatives on the growth of cancer cell lines was evaluated according to the procedure adopted by the National Cancer Institute for *in vitro* anticancer drug screening that uses the protein-binding dye sulforhodamine B to estimate cell growth. Briefly, cells in their log phase of growth were harvested, counted and seeded (10^4 cells/well in 100 μL medium) in 96-well microtitre plates. After 24 h of incubation at 37 °C and 5% CO_2 to allow cell attachment, cultures were treated with varying concentrations (0.1–100 μM) of test samples made with 1:10 serial dilutions. Four replicate wells were set up for each experimental condition. Test samples were left in contact with the cells for 48 h under same conditions. Thereafter cells were fixed with 50% chilled TCA and kept at 4 °C for 1 h, washed and air-dried. Cells were stained with sulforhodamine B dye. The adsorbed dye was dissolved in Tris-buffer and the plates were gently shaken for 10 min on a mechanical shaker. The optical density (OD) was recorded on ELISA reader at 540 nm. The cell growth was calculated by subtracting mean OD value of the respective blank from the mean OD value of experimental set. Percentage of growth in the presence of test material was calculated

considering the growth in the absence of any test material as 100% and the results are reported in terms of IC_{50} values. Podophyllotoxin and etoposide were taken as positive controls. A comparison of IC_{50} values for the tested compounds with the standards is provided in Table 2.

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