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Synthesis and biological evaluation of new 4β-anilino- and 4β-imido-substituted podophyllotoxin congeners

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Abstract—A series of C-4-anilino- and C-4-imido-substituted new podophyllotoxin congeners have been designed, synthesized, and evaluated for their cytotoxicity and DNA topoisomerase-II (topo-II) inhibition potential. Some of these compounds have exhibited promising in vitro anticancer and topo-II inhibition activity.

1. Introduction

Podophyllotoxin (**1**) is a naturally occurring aryltetralin lignan obtained from a number of plant species of the Podophyllum family.[1,2] It has cytotoxic properties and is known as an antimicrotubule agent acting at the colchicine-binding site on tubulin. However, the semisynthetic derivatives of podophyllotoxin, namely etoposide[3,4] (**2**) and teniposide[5] (**3**), inhibit DNA topoisomerase-II (topo-II) by stabilizing the covalent topo-II DNA cleavable complex.[6] Both these compounds are in clinical usage for the treatment of various cancers including small cell lung cancer, testicular carcinoma, and lymphoma.[3,7] Their therapeutic use has encountered certain limitations such as acquired drug resistance and poor water solubility. To overcome such problems, extensive synthetic efforts have been carried out by a number of researchers. This led to the development of etopophos (**4**), NK 611 (**5**), and GL 331 (**6**). Etopophos is a water-soluble prodrug which readily converts into the active drug and exhibits similar biological profile to that of etoposide.[7b,7c] The other analogs of podophyllotoxin like NK 611 (**5**) and GL 331 (**6**) are in different stages of clinical studies.

During the course of preparing new C-4-nonsugar derivatives of podophyllotoxin, it has been observed that the N-linked congeners like GL 331 (**6**) and NPF (**7**) have exhibited improved cytotoxicity and topo-II inhibition activity (Fig. 1).[4a,5,8] A recent study on molecular-area-oriented chemical modifications of podophyllotoxin has revealed certain structural features that are critical for the topo-II inhibition.[9] The comparative molecular field analysis [8b,9] model further demonstrated that bulky substituents at C-4 might be favorable for topo-II inhibition (Fig. 2). Recently, we have been involved in the development of new synthetic procedures[10] for the podophyllotoxin-based compounds and also in the design and synthesis of new analogs of podophyllotoxin as potential anticancer agents.[11] In conjunction with these efforts, it was of interest to prepare 4β-(2″-benzoyl) anilino- and 4β-imido-substituted podophyllotoxin analogs[12] with the objective to enhance their topo-II inhibition as well as in vitro anticancer activity particularly by increasing the bulkiness at the C-4 position.

2. Chemistry

The synthesis of C-4β-substituted analogs of podophyllotoxin has been carried out from podophyllotoxin (**1**). The key intermediate for the preparation of the new analogs is 4β-bromopodophyllotoxin/4β-bromo-4′-demethylpodophyllotoxin (**8**) that was obtained by a

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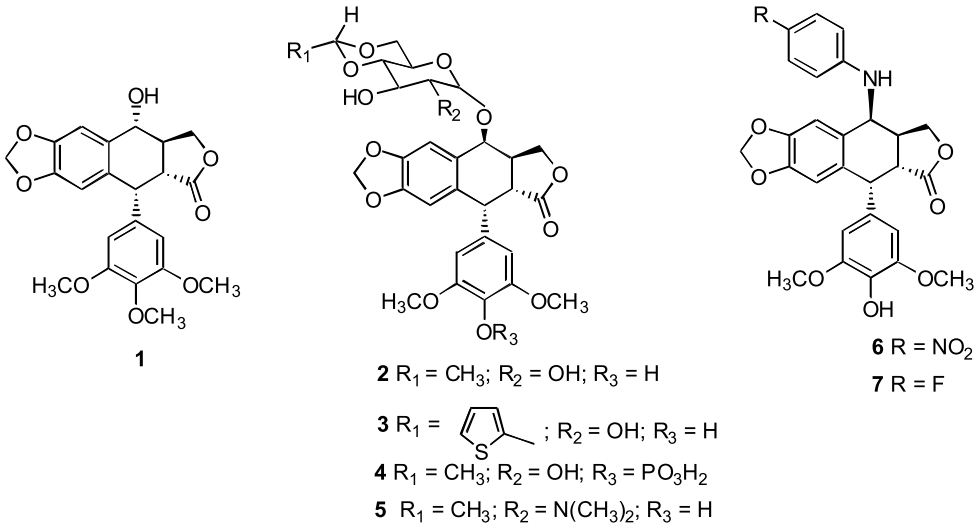


Figure 1. Structures of podophyllotoxin (**1**), etoposide (**2**), teniposide (**3**), etopophos (**4**), NK 611 (**5**), GL 331 (**6**), and NPF (**7**).

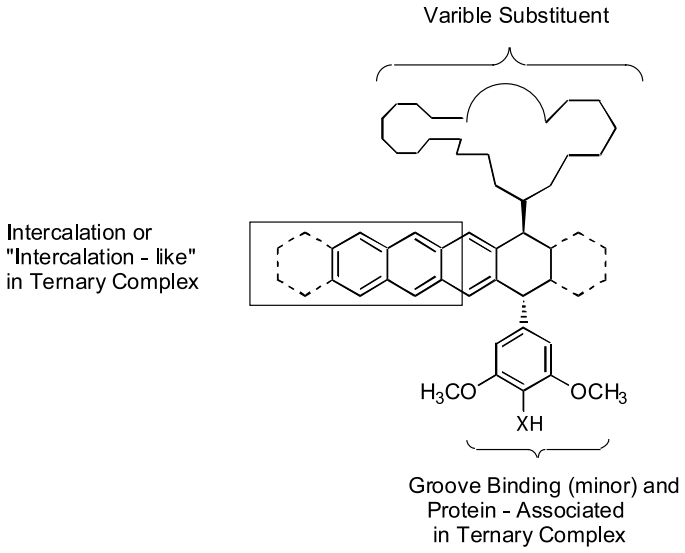


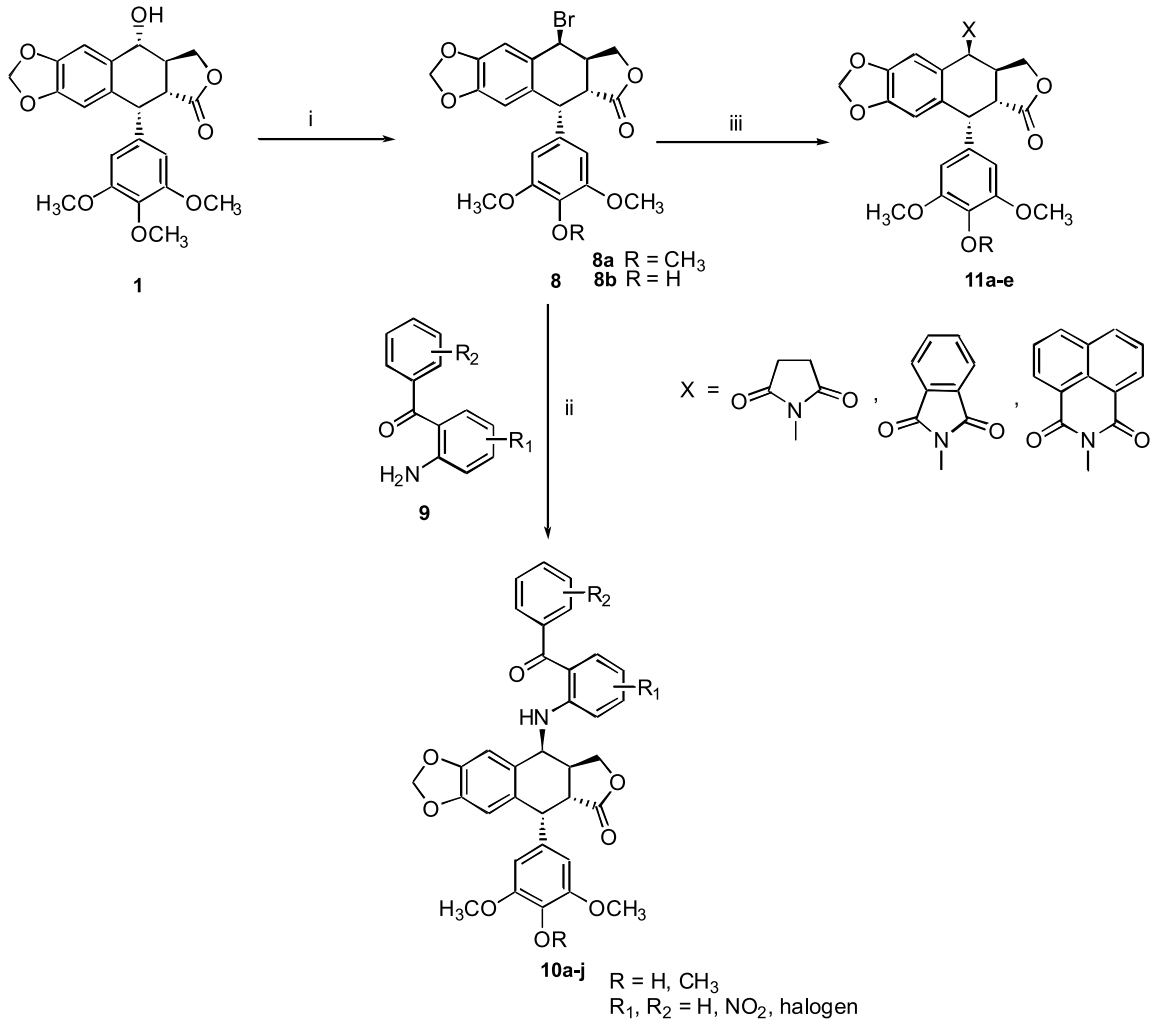
Figure 2. Pharmacophore model of podophyllotoxin analogs.

previously reported method[13] employing HBr in dichloromethane. The intermediate (**8**) thus obtained has been utilized for the synthesis of the final target molecules **10a–j** and **11a–e**. Compounds **10a–j** are obtained by coupling of **8** with substituted 2-aminobenzophenones employing Bu_4NI, and NEt_3 in tetrahydrofuran (THF) at room temperature in good yields (40–70%), whereas coupling of **8** with various imides like succinimide, phthalimide, and naphthalimide using Bu_4NI, and NEt_3 in THF at room temperature gives the corresponding 4β-imido-substituted podophyllotoxin analogs (**11a–e**) in 30% yield (Scheme 1).

3. Biological evaluation

These 4β-anilino- and 4β-imido-substituted podophyllotoxin analogs have been tested for their cytotoxic activities against six human cancer cell lines that comprise of DU145, HT29, MCF7, MCF7ADR, NCIH460, and U251. The screening procedure is based on the routine method adopted by NCI as described in the previous report.[11a] These analogs of podophyllotoxin (**10a–j** and **11a–d**) have exhibited an interesting profile of in vitro anticancer activity. The compound **10j** having C1 and F substituents in 4β-(2″-benzoyl) anilino-substituted series is highly potent against all the cancer cell lines and similarly **11d** has exhibited very high in vitro anticancer activity (Tables 1 and 2). However, the cytotoxicity (IC_{50} in KB cell lines) of compound **6** has been reported[5] as 1.3 μM. On the basis of the in vitro anticancer activity, **10j** has been investigated for its in vivo activity but unfortunately this compound has problems of bioavailability.

Some representative compounds in the 4β-anilino series (**10a**, **10c**, **10e**, and **10g**) have been evaluated in the 60 cancer cell line assay of NCI (Table 3). Interestingly, both the 4′-*O*-methyl and the 4′-*O*-demethyl analogs exhibited good in vitro anticancer activity. As demonstrated by the mean graph pattern for compound **10c**



Scheme 1. Reagents and conditions: (i) HBr, CH_2Cl_2, 0 °C, 45 min (**8a**); HBr, CH_2Cl_2, 0 °C, rt, 48 h (**8b**); (ii) Bu_4NI, Et_3N, THF, rt; (iii) succinimide, phthalimide, naphthalimide, Bu_4NI, Et_3N, THF, rt.

(Fig. 3), it is seen that it exhibits not only significant activity but also more sensitivity for certain cancer cell lines. Therefore, from the structure–activity relationship point of view, it is not clear about the role played by this functionality. In literature, it has been observed that demethylation of the 4′-methoxy moiety eliminates antimitotic activity but there is no clarity about its relationship for the topo-II inhibition activity. For the podophyllotoxin lignans, DNA topo-II is the pharmacological target of clinical relevance; therefore, some representative compounds have been evaluated for its inhibition. Most of the compounds (e.g., **10b**, **10g**, **10j**, and **11d**) exhibited comparable in vitro inhibition of topo-II catalytic activity to m-AMSA and the results are illustrated in Figure 4. The topo-II-mediated relaxation assay performed is according to the previous procedure.[14]

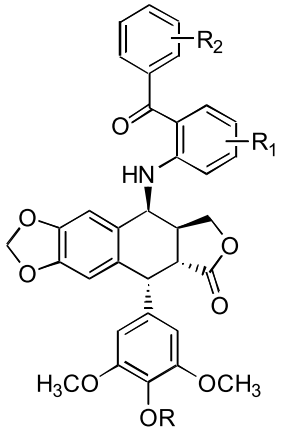
4. Conclusion

In summary, the newly designed and synthesized C-4-modified podophyllotoxin congeners exhibit promising in vitro cytotoxic activities in a number of human cancer cell lines. Two of the compounds from this series exhibited interesting DNA topo-II inhibition activity, suggesting that these new molecules also exhibit biological activity based on this mechanism similar to the etoposide prototypes. Further, these results agree with the hypothesis that the bulky substitution at C-4 position can enhance the activity profile for this class of compounds. This investigation suggests that these podophyllotoxin congeners irrespective of their interaction with DNA possess potential in vitro anticancer activity. Moreover, efforts are in progress to improve the bioavailability of these compounds.

5. Experimental

The NMR spectra are recorded on Varian Gemini 200 MHz spectrometer, using TMS as an internal reference. IR spectra are recorded on Perkin-Elmer model 683 or 1310 spectrometer with sodium chloride optics. Mass spectra are recorded on CEC-21-100B, Finnigan Mat 1210, or Micromass 7070 spectrometer operating at 70 eV using a direct inlet system. Optical rotations are measured on Jasco Dip 360 digital polarimeter.

Table 1. Average GI_{50} values of the 4β-anilino-substituted analogs of podophyllotoxin in six cell lines

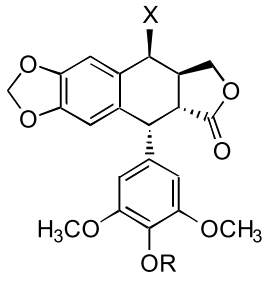


10a-j

Compound	R	R_1	R_2	GI_{50} (μM)
Etoposide[a] **(2)**	—	—	—	0.80–116
10a	CH_3	H	H	0.04–0.5
10b	H	H	H	15–382
10c	CH_3	4-Cl	2-Cl	0.059–0.876
10d	H	4-Cl	2-Cl	0.10–0.24
10e	CH_3	4-NO_2	H	<10 nm–0.28
10f	H	4-NO_2	H	0.01–0.24
10g	CH_3	4-Cl	H	0.07–1.10
10h	H	4-Cl	H	14–270
10i	CH_3	4-Cl	2-F	0.14–0.30
10j	H	4-Cl	2-F	0.004–0.10

[a] Values from NCI database.

Table 2. Average GI_{50} values of the 4β-imido-substituted analogs of podophyllotoxin in six cell lines



11a-e

Compound	X	R	GI_{50} (μM)
Etoposide[a] **(2)**	—	—	0.80–116
11a	Succinimido	H	0.16–0.50
11b	Succinimido	CH_3	0.03–0.40
11c	Phthalimido	H	0.10–0.24
11d	Phthalimido	CH_3	0.004–0.02
11e	Naphthalimido	CH_3	—

[a] Values from NCI database.

Melting points are determined on an electrothermal melting point apparatus and are uncorrected. TLC is performed with E. Merck precoated silica gel plates (60F-254) with iodine as a developing agent. Acme, India silica gel (100–200 mesh) is used for column chromatography.

Table 3. In vitro cytotoxicity of compounds **10a**, **10c**, **10e**, and **10g** in selected human cancer cell lines

Cancer panel/cell line	GI_{50} (μM)			
	10a	**10c**	**10e**	**10g**
Leukemia				
CCRF-CEM	0.36	0.04	—	0.41
SR	0.34	0.03	0.15	0.27
Nonsmall cell lung				
NCI-H522	0.25	0.09	0.14	0.15
Colon				
KM 12	0.25	0.03	0.25	0.20
CNS				
SF-295	0.34	0.05	0.27	0.36
SF-539	0.25	0.02	0.18	0.26
Renal				
ACHN	0.35	0.02	0.21	0.48
Breast				
HS-578T	0.34	0.02	0.51	—

5.1. Chemistry

5.1.1. 4β-Bromopodophyllotoxin (8a). To a stirred solution of podophyllotoxin (**1**) (1.1 g, 2.65 mmol) in dichloromethane and 1 mL of ether, molecular sieves (4Å) powder was added. Dry HBr gas was bubbled through the solution to saturation (about 45 min) at 0 °C. Later, the solution was filtered (to remove molecular sieves powder) and the solvent was removed under reduced pressure. The product obtained in 60% yield and utilized for next step without purification.

5.1.2. 4′-*O*-Demethyl-4β-bromo-4-desoxypodophyllotoxin (8b). To a stirred solution of podophyllotoxin (**1**) (1.1 g, 2.65 mmol) in dichloromethane and 1 mL of ether, molecular sieves (4Å) powder was added. Dry HBr gas was bubbled through the solution to saturation (about 45 min) at 0 °C. The reaction mixture was allowed to stir for 48 h at room temperature and filtered to remove the molecular sieves powder. The filtrate was evaporated under reduced pressure to leave the residue, which was used for next step without further purification.

5.1.3. 4β-1″-[2″-(Benzoyl) anilino]-4-desoxypodophyllotoxin (10a). 4β-Bromo-4-desoxypodophyllotoxin (0.1 g, 0.21 mmol) was reacted with 2-aminobenzophenone (0.045 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 4 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.8:0.2) as an eluent.

Yield 60%, mp 140–142 °C; $[\alpha]_D^{25}$ −112 (*c* 0.1, $CHCl_3$); IR (KBr) 3400, 2900, 1780, 1500, 1480, 1410, 1300, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.82 (d, 1H), 7.50 (m, 7H), 6.80 (s, 1H), 6.75 (d, 1H), 6.68 (d, 1H), 6.55 (s, 1H), 6.35 (s, 2H), 5.96 (d, 2H), 4.92 (m, 1H), 4.65 (d, 1H), 4.35 (t, 1H), 3.96 (t, 1H), 3.82 (d, 9H), 3.20 (q, 1H), 3.05 (m, 1H); MS 593 ($M^{+\cdot}$). Anal. Calcd for $C_{35}H_{31}NO_8$: C, 70.82; H, 5.26; N, 2.36. Found: C, 70.85; H, 5.28; N, 2.39.

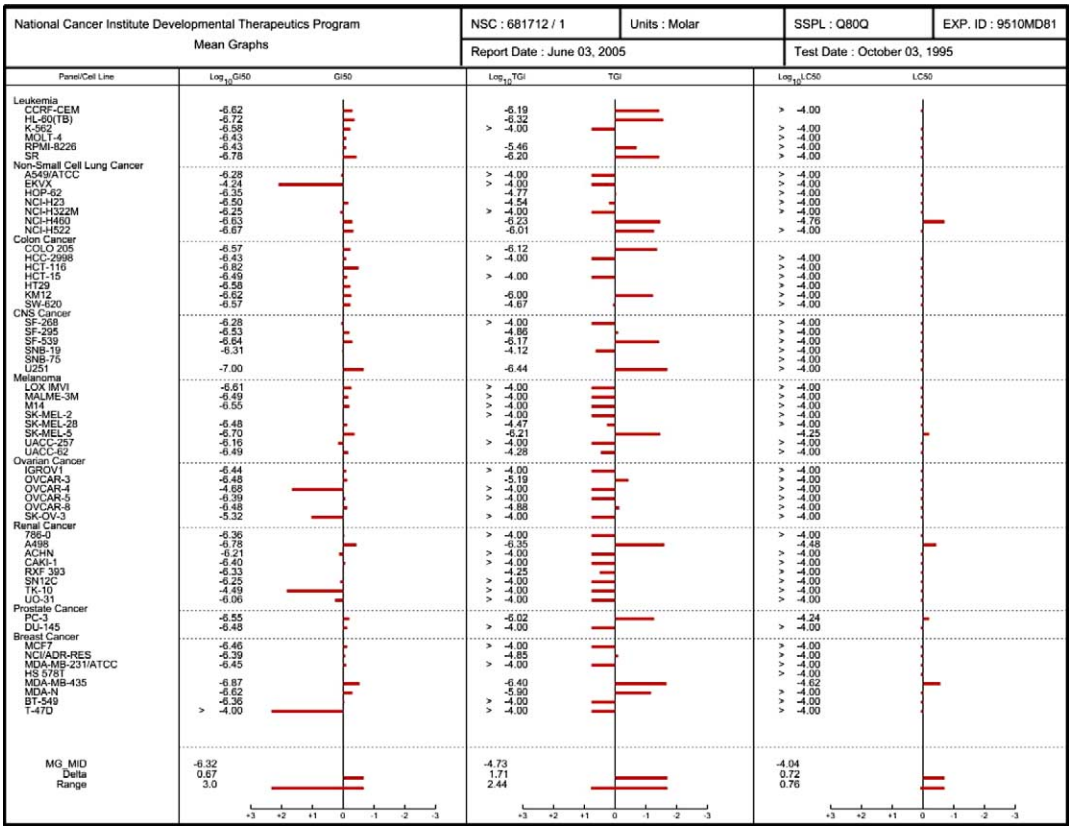


Figure 3. $Log_{10}GI_{50}$, $log_{10}TGI$, and $log_{10}LC_{50}$ data from the NCI 60 cell line screen for the compound **10c**.

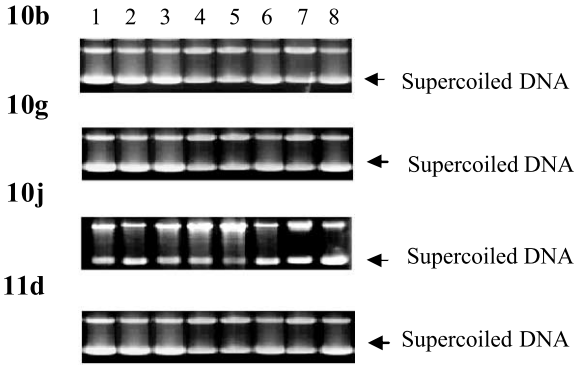


Figure 4. Inhibition of DNA topoisomerase-II catalyzed relaxation of supercoiled DNA by compounds **10b**, **10g**, **10j**, and **11d**. Lanes 1–5: Compounds **10b**, **10g**, **10j**, and **11d** (200, 100, 50, 25, and 10 μM, respectively); lane 6: m-AMSA (200 μM) postive control; lane 7: Topo-II a negative control; lane 8: 0.6 μg PRYG plasmid DNA.

5.1.4. 4′-*O*-Demethyl-4β-1″-[2″-(benzoyl) anilino]-4-desoxypodophyllotoxin (10b). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.1 g, 0.21 mmol) was reacted with 2-aminobenzophenone (0.045 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 4 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.8:0.2) as an eluent.

Yield 50% mp 154–156 °C; $[\alpha]_D^{25}$ 111 (*c* 1.1, $CHCl_3$); IR (KBr) 3550, 3400, 2900, 1750, 1650, 1500, 1480, 1410, 1300, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.85 (d, 1H), 7.50 (m, 7H), 6.80 (s, 1H), 6.75 (d, 1H), 6.68 (d, 1H), 6.55 (s, 1H), 6.35 (s, 2H), 5.96 (d, 2H), 5.38 (s, 1H), 4.92 (m, 1H), 4.65 (d, 1H), 4.35 (t, 1H), 3.96 (t, 1H), 3.82 (s, 6H), 3.20 (q, 1H), 3.05 (m, 1H); MS 579 ($M^{+\cdot}$). Anal. Calcd for $C_{34}H_{29}NO_8$: C, 70.46; H, 5.04; N, 2.42. Found: C, 70.50; H, 5.08; N, 2.39.

5.1.5. 4β-1″-[2″-(2-Chlorobenzoyl)-4″-chloroanilino]-4-desoxypodophyllotoxin (10c). 4β-Bromo-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with 2-amino-2′,5′-dichlorobenzophenone (0.06 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 5 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.7:0.3) as an eluent.

Yield 64% mp 142–145 °C; $[\alpha]_D^{25}$ −84 (*c* 0.87, $CHCl_3$); IR (KBr) 3350, 2900, 1760, 1640, 1550, 1480, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.10 (d, 1H), 7.40 (m, 5H), 7.20 (d, 1H), 6.78 (s, 1H), 6.75 (d, 1H), 6.52 (s, 1H), 6.35 (s, 2H), 5.96 (d, 2H), 4.97 (m, 1H), 4.65 (d, 1H), 4.35 (t, 1H), 3.90 (t, 1H), 3.77 (d, 9H), 3.20 (q, 1H), 3.10 (m, 1H); MS 663 ($M^{+\cdot}$). Anal. Calcd for $C_{35}H_{29}Cl_2NO_8$: C, 63.45; H, 4.41; N, 2.11. Found: C, 63.48; H, 4.44; N, 2.14.

5.1.6. 4′-*O*-Demethyl-4β-1″-[2″-(2-chlorobenzoyl)-4″-chloroanilino]-4-desoxypodophyllotoxin (10d). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with 2-amino-2′,5′-dichlorobenzophenone (0.06 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in tetrahydrofuran at room temperature for 5 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.7:0.3) as an eluent.

Yield 70% mp 151–153 °C; $[\alpha]_D^{25}$ −91 (*c* 0.93, $CHCl_3$); IR (KBr) 3320, 2900, 1760, 1650, 1550, 1480, 1410, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.10 (d, 1H), 7.40 (m, 5H), 7.20 (d, 1H), 6.77 (s, 1H), 6.70 (d, 1H), 6.30 (s, 2H), 5.96 (d, 2H), 5.40 (s, 2H), 4.90 (m, 1H), 4.65 (d, 1H), 4.30 (t, 1H), 4.10 (t, 1H), 3.80 (s, 6H), 3.20 (q, 1H), 3.10 (m, 1H); MS 649 ($M^{+\cdot}$). Anal. Calcd for $C_{34}H_{27}Cl_2NO_8$: C, 62.97; H, 4.20; N, 2.16. Found: C, 62.94; H, 4.23; N, 2.14.

5.1.7. 4β-1″-[2″-(Benzoyl)-4″-nitroanilino]-4-desoxypodophyllotoxin (10e). 4β-Bromo-4-desoxypodophyllotoxin (0.1 g, 0.21 mmol) was reacted with 2-amino-5-nitrobenzophenone (0.056 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 8 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.5:0.5) as an eluent.

Yield 40%, mp 163–167 °C; $[\alpha]_D^{25}$ −85 (*c* 1.2, $CHCl_3$); IR (KBr) 3450, 2950, 1740, 1650, 1550, 1480, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.50 (d, 1H), 8.57 (d, 1H), 8.32 (q, 1H), 7.60 (m, 4H), 6.75 (d, 1H), 6.70 (s, 1H) 6.60 (d, 1H), 6.40 (d, 1H), 6.30 (s, 2H), 6.00 (d, 2H), 5.05 (m, 1H), 4.70 (d, 1H), 4.40 (t, 1H), 3.90 (t, 1H), 3.80 (d, 9H), 3.15 (d, 1H), 2.95 (m, 1H); MS 638 ($M^{+\cdot}$). Anal. Calcd for $C_{35}H_{30}N_2O_{10}$: C, 65.83; H, 4.73; N, 4.39. Found: C, 65.85; H, 4.76; N, 4.41.

5.1.8. 4′-*O*-Demethyl-4β-1″-[2″-(benzoyl)]-4″-nitroanilino]-4-desoxypodophyllotoxin (10f). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.1 g, 0.21 mmol) was reacted with 2-amino-5-nitro-benzophenone (0.056 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 8 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.5:0.5) as an eluent.

Yield 38%, mp 169–171 °C; $[\alpha]_D^{25}$ −89 (*c* 1.0, $CHCl_3$); IR (KBr) 3560, 3400, 2900, 1740, 1650, 1500, 1480, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.47 (d, 1H), 8.55 (d, 1H), 8.30 (q, 1H), 7.60 (m, 4H), 6.80 (d, 1H), 6.70 (s, 1H), 6.55 (s, 1H) 6.35 (d, 1H), 6.30 (s, 2H), 6.00 (d, 2H), 5.87 (s, 1H), 5.00 (m, 1H), 4.65 (d, 1H), 4.30 (m, 2H), 3.80 (d, 6H), 3.15 (d, 1H), 3.00 (m, 1H); MS 624 ($M^{+\cdot}$). Anal. Calcd for $C_{34}H_{28}N_2O_{10}$: C, 65.38; H, 4.52; N, 4.49. Found: C, 65.35; H, 4.56; N, 4.51.

5.1.9. 4β-1″-[2″-(Benzoyl)-4″- chloroanilino]-4-desoxypodophyllotoxin (10g). 4β-Bromo-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with 2-amino-5-chlorobenzophenone (0.053 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 6 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.7:0.3) as an eluent.

Yield 56% mp 139–142 °C; $[\alpha]_D^{25}$ −103 (*c* 0.93, $CHCl_3$); IR (KBr) 3350, 2900, 1780, 1660, 1500, 1480, 1410, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.72 (d, 1H), 7.60 (m, 7H), 6.80 (s, 1H), 6.75 (d, 1H), 6.55 (s, 1H), 6.35 (s, 2H), 5.98 (d, 2H), 4.95 (m, 1H), 4.65 (d, 1H), 4.40 (t, 1H), 3.95 (t, 1H), 3.80 (d, 9H), 3.20 (q, 1H), 3.10 (m, 1H); MS 628 ($M^{+\cdot}$). Anal. Calcd for $C_{35}H_{30}ClNO_8$: C, 66.93; H, 4.81; N, 2.23. Found: C, 66.94; H, 4.84; N, 2.26.

5.1.10. 4′-*O*-Demethyl-4β-1″-[2″-(benzoyl)]-4″-chloroanilino]-4-desoxypodophyllotoxin (10h). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.1 g, 0.21 mmol) was reacted with 2-amino-5-chlorobenzophenone (0.053 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 6 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.7:0.3) as an eluent.

Yield 50%, mp 146–149 °C; $[\alpha]_D^{25}$ −105 (*c* 0.97, $CHCl_3$); IR (KBr) 3500, 3360, 2900, 1750, 1640, 1500, 1480, 1230 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.68 (d, 1H), 7.52 (m, 7H), 6.72 (s, 1H), 6.65 (d, 1H), 6.50 (s, 1H) 6.30 (s, 2H), 5.96 (d, 2H), 5.35 (s, 1H), 4.85 (m, 1H), 4.60 (d, 1H), 4.30 (t, 1H), 3.85 (d, 1H), 3.80 (s, 6H), 3.10 (q, 1H), 3.00 (m, 1H); MS 614 ($M^{+\cdot}$). Anal. Calcd for $C_{34}H_{28}ClNO_8$: C, 66.50; H, 4.60; N, 2.28. Found: C, 66.54; H, 4.57; N, 2.26.

5.1.11. 4β-1″-[2″-(2-Fluorobenzoyl)-4″-chloroanilino]-4-desoxypodophyllotoxin (10i). 4β-Bromo-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with 2-amino-5-chloro-2′-fluorobenzophenone (0.057 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 5 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.7:0.3) as an eluent.

Yield 68% mp 123–128 °C; $[\alpha]_D^{25}$ −89 (*c* 1.0, $CHCl_3$); IR (KBr) 3400, 2950, 1760, 1650, 1500, 1480, 1300, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.10 (d, 1H), 7.45 (m, 6H), 6.80 (s, 1H), 6.75 (d, 1H), 6.55 (s, 1H), 6.35 (s, 2H), 6.00 (d, 2H), 4.95. (m, 1H), 4.70 (d, 1H), 4.40 (t, 1H), 3.95 (t, 1H), 3.82 (d, 9H), 3.20 (q, 1H), 3.10 (m, 1H); MS 646 ($M^{+\cdot}$). Anal. Calcd for $C_{35}H_{29}ClFNO_8$: C, 65.07; H, 4.52; N, 2.17. Found: C, 65.09; H, 4.55; N, 2.19.

5.1.12. 4′-*O*-Demethyl-4β-1″-[2″-(2-fluorobenzoyl)-4″-chloroanilino]-4-desoxypodophyllotoxin (10j). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with 2-amino-5-chloro-2-fluorobenzophenone (0.057 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 5 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using chloroform/methanol (9.7:0.3) as an eluent.

Yield 60% mp 164–167 °C; $[\alpha]_D^{25}$ −85 (*c* 1.01, $CHCl_3$); IR (KBr) 3520, 3440, 2900, 1750, 1650, 1500, 1480, 1300, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.05 (d, 1H), 7.48 (m, 6H), 6.80 (s, 1H), 6.75 (d, 1H), 6.52 (s, 1H), 6.35 (s, 2H), 6.00 (d, 2H), 5.10 (s, 1H), 4.98 (m, 1H), 4.70 (d, 1H), 4.40 (t, 1H), 3.95 (t, 1H), 3.82 (s, 6H), 3.20 (q, 1H), 3.10 (m, 1H); MS 632 ($M^{+\cdot}$). Anal. Calcd for $C_{34}H_{27}ClFNO_8$: C, 64.61; H, 4.31; N, 2.22. Found: C, 64.64; H, 4.35; N, 2.18.

5.1.13. 4′-*O*-Demethyl-4β-succinimido-4-desoxypodophyllotoxin (11a). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with succinimide (0.024 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 6 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using ethyl acetate/hexane (7:3) as an eluent.

Yield 30% mp 197–201 °C; $[\alpha]_D^{25}$ −104 (*c* 0.05, EtOAc:-MeOH); IR (KBr) 3500, 2880, 1800, 1630, 1530, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.85 (s, 1H), 6.55 (s, 1H), 6.25 (s, 2H), 6.00 (d, 2H), 5.35 (s, 1H), 4.85. (d, 1H), 4.58 (d, 1H), 4.35 (d, 2H), 3.78 (s, 6H), 3.24 (q, 1H), 2.75 (s,4H), 2.30 (m, 1H); MS 481 ($M^{+\cdot}$). Anal. Calcd for $C_{25}H_{23}NO_9$: C, 62.37; H, 4.82; N, 2.91. Found: C, 62.35; H, 4.80; N, 2.89.

5.1.14. 4β-Succinimido-4-desoxypodophyllotoxin (11b). 4β-Bromo-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with succinimide (0.023 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 6 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using ethyl acetate/hexane (7:3) as an eluent.

Yield 30% mp 168–172 °C; $[\alpha]_D^{25}$ −97 (*c* 0.6, EtOAc:-MeOH, 1:1); IR (KBr) 2850, 1800, 1620, 1500, 1480, 1410, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.85, (s, 1H), 6.52 (s, 1H), 6.25 (s, 2H), 5.90 (d, 2H), 4.85 (d, 1H), 4.55 (d, 1H), 4.35 (d, 2H), 3.75 (d, 9H), 3.25 (q, 1H), 2.75 (s, 4H), 2.40 (m, 1H); MS 495 ($M^{+\cdot}$). Anal. Calcd for $C_{26}H_{25}NO_9$: C, 63.03; H, 5.09; N, 2.83. Found: C, 63.06; H, 5.12; N, 2.87.

5.1.15. 4β-Phthalimido-4-desoxypodophyllotoxin (11c). 4β-Bromo-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with phthalimide (0.033 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 5 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using ethyl acetate/hexane (8:2) as an eluent.

Yield 30% mp 135–139 °C; $[\alpha]_D^{25}$ −69 (*c* 1.1, $CHCl_3$); IR (KBr) 2940, 1760, 1740, 1530, 1410, 1250 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.85 (m, 2H), 7.75 (m, 2H), 6.75 (s, 1H), 6.55 (s, 1H), 6.20 (s, 2H), 5.97 (d, 2H), 4.57 (d, 1H), 4.33. (d, 1H), 4.27 (d, 2H), 3.75 (d, 9H), 3.35 (q, 1H), 2.80 (m, 1H); MS 544 ($M^{+\cdot}$). Anal. Calcd for $C_{30}H_{25}NO_9$: C, 66.29; H, 4.64; N, 2.58. Found: C, 66.26; H, 4.62; N, 2.61.

5.1.16. 4′-*O*-Demethyl-4β-phthalimido-4-desoxypodophyllotoxin (11d). 4β-Bromo-4′-*O*-demethyl-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with phthalimide (0.034 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 5 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using ethyl acetate/hexane (8:2) as an eluent.

Yield 30% mp 156–160 °C; $[\alpha]_D^{25}$ −58 (*c* 1.0, $CHCl_3$); IR (KBr) 3300, 2940, 1760, 1740, 1530, 1410, 1250 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.85 (m, 2H), 7.75 (m, 2H), 6.78 (s, 1H), 6.55 (s, 1H), 6.22 (s, 2H), 5.95 (d, 2H), 5.32 (s, 1H), 4.55. (d, 1H), 4.35 (d, 1H), 4.29 (d, 2H), 3.80 (s, 6H), 3.35 (q, 1H), 2.85 (m, 1H); MS 530 ($M^{+\cdot}$). Anal. Calcd for $C_{29}H_{23}NO_9$: C, 65.78; H, 4.38; N, 2.65. Found: C, 65.74; H, 4.32; N, 2.61.

5.1.17. 4β-Naphthalimido-4-desoxypodophyllotoxin (11e). 4β-Bromo-4-desoxypodophyllotoxin (0.10 g, 0.21 mmol) was reacted with naphthalimide (0.056 g, 0.23 mmol) in the presence of Et_3N (0.032 g, 0.32 mmol) and $Bu_4N^+I^-$ (0.015 g, 0.042 mmol) in dry tetrahydrofuran at room temperature for 7 h. After completion of the reaction, the solvent was removed in vacuo. The residue was subjected to silica gel column chromatography using ethyl acetate/hexane (8:2) as an eluent.

Yield 30% mp 132–136 °C; $[\alpha]_D^{25}$ −64 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.72 (m, 2H), 7.55 (m, 2H), 6.8 (s, 1H), 6.55 (t, 2H), 6.23 (s, 2H), 6.10 (s, 1H), 5.97 (d, 2H), 4.58. (d, 1H), 4.35 (d, 1H), 4.28 (t, 2H), 3.76 (d, 9H), 3.35 (dd, 1H), 2.85 (m, 1H). MS 593 ($M^{+\cdot}$). Anal. Calcd for $C_{34}H_{27}NO_9$: C, 68.80; H, 4.58; N, 2.36. Found: C, 68.84; H, 4.53; N, 2.40.

5.2. Biological evaluation

5.2.1. In vitro evaluation of cytotoxic activity. In routine screening, each agent is tested over a broad concentration range (10-fold dilutions starting from >100 μM to ~10 nM) against six human cancer cell lines comprised of different tumor types. Standard compound doxorubicin is tested in each assay as a positive control. The cells are maintained in growing condition in RPMI 1640

medium containing 10% fetal calf serum and incubated at 37 °C under 5% CO_2 atmosphere. All cell lines are inoculated onto a series of standard 96-well microtiter plate on day 0, followed by 24 h incubation in the absence of test compound. The inoculation densities used in this screen are as per the procedure of Monks et al.[15] All NCEs are dissolved in DMSO and diluted further in culture medium. An aliquot of each dilution is added to the growing cells in 96-well plates and incubated for 48 h. After incubation, the assay is terminated by adding 50 μL of trichloro acetic acid and incubating at 4 °C for 30 min. The precipitated cells are washed and stained with sulphorhodamine B dye for 30 min and the excess dye is washed off with acetic acid. Adsorbed dye is solublized in Tris base (alkaline pH) and quantitated by measuring the OD at 490 nm in an ELISA reader. GI_{50} (concentration that inhibits the cell growth by 50%) is calculated according to the method of Boyd.[16]

5.2.2. Immunoprecipitation of topoisomerase-II α. Brain extracts (100 μg total protein) prepared from cerebellum of embryos (E11, E18, and 1-day-old) and whole brain, cerebellum, cerebral cortex, and midbrain regions of the young, adult, and old age groups were taken in Eppendorf tubes for immunoprecipitation and topo-II α or β antibody (1:1000 dilution in immunoprecipitation buffer containing 100 mM Tris–HCl, pH 8, 750 mM NaCl, 2 mM EDTA, 1 mM PMSF, and 0.75% Nonidet) was added to each sample. The antigen–antibody mixture was incubated at room temperature for 1 h and 25 μl of 6% protein An agarose beads was added. The beads were incubated at 4 °C for 15 min, spun down and the supernatant was removed. The protein An agarose beads were washed twice with 0.5% Triton X-100 in PBS. The beads were directly used for monitoring the relaxation activity of topo-II.[17]

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References and notes

1. Lee, K. H. Human Medicinal Agents from Plants. In *American Chemical Society, Symposium Series*; Kinghorn, A. D., Balandrin, M., Eds.1993; American Chemical Society: Washington, DC, p 170.
2. MacRae, W. D.; Hudson, J. B.; Towers, B. H. N. *Planta Med.* **1993**, *55*, 531.
3. Jardine, I. In *Anti-Cancer Agents Based on Natural Products Models*; Cassady, J. M., Doures, J., Eds.; Academic: New York, 1980, p 319.
4. (a) Lee, K. H.; Beers, S. A.; Mori, M.; Wang, Z.; Kuo, Y.; Li, L.; Liu, S.; Cheng, Y.; Han, F.; Cheng, Y. *J. Med. Chem.* **1990**, *33*, 1364; (b) Stahelin, H.; Von Wartburg, A. *Prog. Drug. Res.* **1989**, *33*, 169; (c) Doyle, T. W. *Etoposide (VP-16) Current Status and New Developments*; Academic: New York, 1984.
5. Wang, Z.; Kuo, Y.; Schnur, D.; Bowen, J. P.; Liu, S.; Hen, F.; Chang, J.; Cheng, Y.; Lee, K. H. *J. Med. Chem.* **1990**, *33*, 2660.
6. Macdonald, T. L.; Lehnert, E. K.; Loper, J. T.; Chow, K. C.; Ross, W. E. In *DNA Topoisomerases in Cancer*; Potmesil, M., Kohn, K. W., Eds.; Oxford University: New York, 1991, p 199, and references cited therein.
7. (a) Issell, B. F. *Cancer Chemother. Pharmacol.* **1982**, *7*, 73; (b) Budman, D. R. *Semin. Oncol.* **1996**, *23*, 8; (c) Greco, F. A.; Hainsworth, J. D. *Semin. Oncol.* **1996**, *23*, 45.
8. (a) Zhou, X. M.; Wang, Z. Q.; Chang, J. Y.; Chen, H. X.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1991**, *34*, 3346; (b) Xiao, Z.; Xiao, Y. D.; Golbraikh, A.; Tropsha, A.; Lee, K. H. *J. Med. Chem.* **2002**, *45*, 2294; (c) VanVilet, D. S.; Tachibana, Y.; Bastow, K. F.; Huang, E. S.; Lee, K. H. *J. Med. Chem.* **2001**, *44*, 1422.
9. Cho, S. J.; Tropsha, A.; Suffness, M.; Cheng, Y. C.; Lee, K. H. *J. Med. Chem.* **1996**, *39*, 1383.
10. (a) Kamal, A.; Gayatri, N. L. *Tetrahedron Lett.* **1996**, *37*, 3359; (b) Kamal, A.; Laxminarayana, B.; Gayatri, N. L. *Tetrahedron Lett.* **1997**, *38*, 6871; (c) Kamal, A.; Damayanthi, Y. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 657; (d) Kamal, A.; Gayatri, N. L.; Rao, N. V. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3097; (e) Kamal, A.; Laxman, N.; Ramesh, G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2059; (f) Kamal, A.; Laxman, N.; Ramesh, G. Indian Patent Nos. 191366 and 192429; (g) Kamal, A.; Kumar, B. A.; Arifuddin, M. *Tetrahedron Lett.* **2004**, *44*, 8457.
11. (a) Kamal, A.; Kumar, B. A.; Arifuddin, M.; Sunanda, G. D. *Bioorg. Med. Chem.* **2003**, *11*, 5135; (b) Kamal, A.; Laxman, E.; Khanna, G. B. R.; Reddy, P. S. M. M.; Rehana, T.; Arifuddin, M.; Neelima, K.; Kondapi, A. K.; Dastidar, S. G. *Bioorg. Med. Chem.* **2004**, *12*, 4197.
12. (a) Kamal, A.; Gayatri, N. L. Indian Patent No. 186131.; (b) Kamal, A.; Reddy, P. S. M. M. PCT Appl. No. PCT/IN02/00091, International Publication No. WO 03/082876 A1.
13. Khun, M.; Keller-Juslen, C.; von Wartburg, A. *Helv. Chem. Acta* **1969**, *52*, 948.
14. Gopal, Y. N. V.; Jayaraju, D.; Kondapi, A. K. *Biochemistry* **1999**, *38*, 4382.
15. Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757.
16. Boyd, M. R.; Paull, K. D. *Drug. Dev. Res.* **1995**, *34*, 91.
17. Kondapi, A. K.; Mulpuri, N.; Mandraju, R. K.; Sasikaran, B.; Rao, K. S. *Int. J. Dev. Neurosci.* **2004**, *22*, 19.