Design and Synthesis of C-Ring Lactone- and Lactam-Based Podophyllotoxin Analogues as Anticancer Agents

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A series of novel podophyllotoxin (PDT) analogues was synthesized in which the lactone moiety was shifted to C ring. Some of the derivatives were also synthesized with modified A ring. Analogues 23 and 25 exhibited potent *in vitro* cytotoxicity against colon cancer ($CaCO_2$) cell line. *p*-Demethylated E-ring analogues exhibited better potency than the corresponding methylated analogues. These analogues showed toxicity comparable to PDT against human erythrocytes albeit at much higher concentrations (100 μg/ml) than their cytotoxicity values.

Key words podophyllotoxin; anticancer; osmotic fragility; MTT assay

Podophyllotoxin (**1**, PDT, Fig. 1) is a well known naturally occurring potent cytotoxic aryltetralin lignan isolated from the genus *Podophyllum* (family: Berberidaceae).[1] Two of its known analogues etoposide (**2**) and teniposide (**3**) show DNA topoisomerase II inhibition activity[2] and are among the frontline antitumour drugs against various cancers, including small cell lung cancer, testicular carcinoma, and Kaposi's sarcoma.[3] A different mode of action of **2** and **3** by minor structural modifications of **1** has been attributed to *p*-demethylation in E-ring, inversion of stereochemistry at C-4 position, and addition of a sugar unit.[4] Furthermore, the replacement of C-4 sugar unit of etoposide with a heteroatom (*O*, *N*, or *S*) linked moieties helped in overcoming the problem of drug resistance to etoposide.[5] Subsequently, a large number of analogues have been reported exhibiting comparable or better activity than **2** and **3**.[6]

The present study was aimed at designing potential cytotoxic PDT analogues taking lactone and lactam moieties as targets. A series of neo-flavonoids was prepared in which the lactone moiety was shifted from ring D to ring C to fulfil the structural requirements (Prototype-I). Alternatively, some lactams (Prototype-II) were also synthesized to replace the oxygen by a nitrogen unit, since *N*-linked congeners were reported to have better activity than *O*-linked and *S*-linked derivatives,[7—10] *e.g.* GL-331, 4′-*O*-demethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (NPF).[11] Some PDT analogues devoid of ring A were also synthesized.

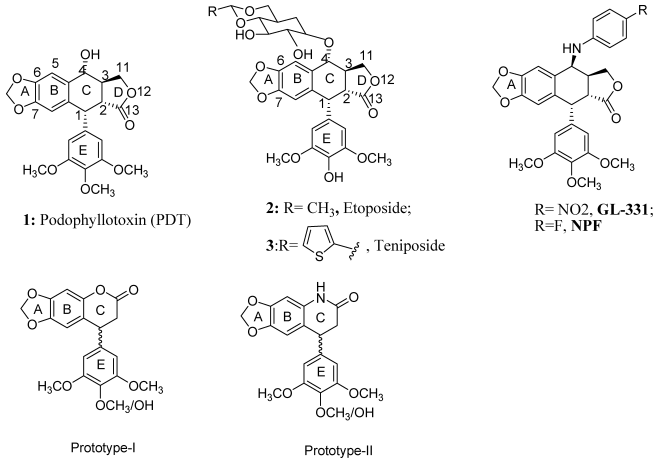

Fig. 1. Structures of Podophyllotoxin (**1**), Etoposide (**2**), Teniposide (**3**), 4β-*N*-Linked Congeners and Prototypes I and II

Chemistry The strategy to synthesize lactones and lactams is depicted in Chart 1 in which piperonal (**4**) was used as starting material. The aldehydic group of piperonal was oxidized to formate ester (**5**) by Baeyer–Villiger oxidation using *m*-chloroperbenzoic acid (*m*-CPBA) in dichloromethane at room temperature in 22% yield.[12] The formate ester (**5**) was hydrolyzed with 5% aqueous methanolic KOH to produce the corresponding phenol (**6**) in 82% yield. However, due to poor yield in the first step, piperonal was replaced with 3,4-methylenedioxyacetophenone (**7**). **7** on Baeyer–Villiger oxidation with *m*-CPBA yielded the corresponding acetate ester (**8**) in 47% yield. On alkaline hydrolysis, **8** yielded 3,4-methylenedioxyphenol (**6**) in 95% yield. **6** was stirred with 3,4,5-trimethoxycinnamic acid methyl ester (**10a**) in trifluoroacetic acid at room temperature for 24 h to produce the desired product (**12**) in 34% yield.[13] Similarly, various other phenols such as 3-methoxyphenol, 3,5-dimethoxyphenol, 3,4-dimethoxyphenol, 6-methoxy-2-naphthol, and estrone were used to produce the corresponding neoflavonoids (**24**—**28**).

To produce lactams as per prototype-II, 3,4,5-trimethoxycinnamic acid (**10b**) was condensed with 3,4-methylenedioxyaniline in presence of *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC), 4-(dimethylamino)pyridine (DMAP) and *N*-hydroxybenzotriazole (HOBt) in *N*,*N*-dimethylformamide (DMF) to yield a corresponding amide (**16**). Amide (**16**) on stirring in trifluoroacetic acid (TFA) for 72 h yielded the corresponding lactam (**18**).[13]

The *p*-demethylated lactone (**13**) was synthesized by stirring 4-hydroxy-3,5-dimethoxycinnamic acid methyl ester (**11**) with phenol (**6**) in TFA. Meanwhile *p*-demethylated lactams were synthesized using 4-acetoxy-3,5-dimethoxycinnamic acid (**15**) to produce corresponding amides (**17**, **21**) and subsequent treatment with TFA to yield *p*-demethylated lactams (**19**, **23**). All the compounds were characterized by IR, NMR, and mass spectrometry.

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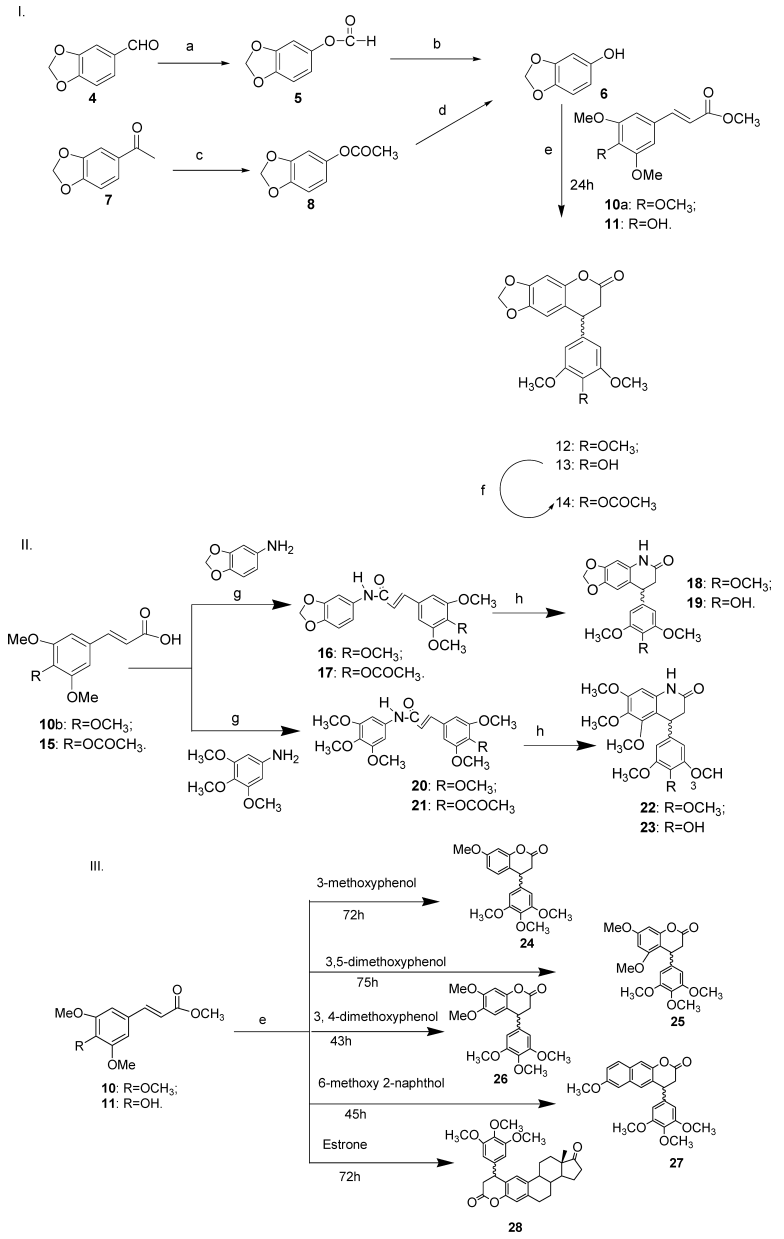

(a) *m*-CPBA, dry DCM, RT, 72 h, 22%; (b) 5% aqueous–methanolic (1 : 1) KOH , 50 °C, 1 h, 82%; (c) *m*-CPBA, dry DCM, RT, 60 h, 47%; (d) 5% aqueous–methanolic (1 : 1) KOH, 50 °C, 1 h, 95%; (e) various phenols, TFA, 24—72 h, 16—60%; (f) pyridine, acetic anhydride, RT, overnight, 98%; (g) EDC–HCl, HOBt, DMF, TEA, reflux, 6 h, 45—62%; (h) TFA, RT, 72 h, 35—66%.

Chart 1

Results and Discussion

All the analogues of PDT based on prototype-I and II were evaluated *in vitro* to establish their cytotoxicity against various human cancer cell lines *i.e.*, $CaCO_2$ (colon), HepG2 (liver), KB (oral), and MCF-7 (hormone-dependent breast) by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay.[14] Paclitaxel (Taxol), PDT and etoposide were used as reference compounds. Although these compounds were screened for various human cancer cell lines, our main interest was in their activity against colon cancer cells. From Table 1 it is clear that among the evaluated 12 analogues, two were highly active (**23**, **25**), five were moderately active (**13**, **14**, **19**, **22**, **24**) and remainder possessed low cytotoxicity against colon cancer cells.

Demethylation or acetylation at *para* position of E-ring increased cytotoxicity in lactones (**12**, **13**, **14**) and lactams (**18**, **19**, **22**, **23**). Thus it is evident that the 3,4,5-trimethoxy system of ring E is not essential for inducing cytotoxicity in these analogues and *p*-demethylated analogues exhibited better activity than their corresponding trimethoxy analogues. Among all these analogues, lactone **25** and lactams **22** and **23** devoid of ring A exhibited higher cytotoxic activity than those possessing ring A. Thus ring A seems not essential to induce cytotoxicity. Since all these analogues exhibited significant anticancer activity even devoid of ring D, ring D also seems not essential for inducing cytotoxicity. However, the requirement of lactone ring cannot be ruled out. There was no significant difference in the cytotoxicity on converting lactone moiety to lactam (**12**, **18**). The inclusion of nitrogen atom in ring C was not beneficial due to insufficient bulkiness, which otherwise is required for better activity at C-4 position of PDT analogues. Overall, the cytotoxicities of ana-

Table 1. Cytotoxicity of Different Compounds against Various Human Cancer Cell Lines by MTT Assay

S. No.	Compound No.	Human cancer cells			
		$CaCO_2$ IC_{50} (μg/ml)	HepG2 IC_{50} (μg/ml)	KB IC_{50} (μg/ml)	MCF-7 IC_{50} (μg/ml)
1	**12**	50	90	50	30
2	**13**	40	60	40	9.0
3	**14**	20	4.5	4.0	70
4	**18**	55	Inactive[a)]	40	20
5	**19**	20	30	6.0	80
6	**22**	20	4.5	10	80
7	**23**	3.0	4.0	1.0	0.8
8	**24**	40	90	5.5	9.0
9	**25**	3.5	45	9.0	80
10	**26**	75	90	50	90
11	**27**	60	80	50	50
12	**28**	55	65	60	6.0
13	Paclitaxel (Taxol)	0.007	n.d.	0.001	0.005
14	Podophyllotoxin	0.001	2.0	8.5	3.5
15	Etoposide	n.d.	n.d.	0.16	4.3
16	Tamoxifen	0.15	0.85	0.005	0.01

a) Inactive (IC_{50} values $>100\mu$g/ml); IC_{50} values mean of three experiments in replicate.

logues **12** and **18** are much less than that of PDT. This indicates that although the presence of ring D is not essential for cytotoxicity, it enhances the activity several fold (PDT). The mechanism of action of these compounds will be further elucidated by biochemical investigations, currently in progress.

Analogues exhibiting potent cytotoxicity were also evaluated by erythrocyte osmotic fragility test to determine their toxicities.[15)] The osmotic fragility profiles of control and *in vitro* PDT analogues (**14**, **19**, **22**, **23**, **25**) treated erythrocytes are shown in Fig. 2. Curcumin was used as positive control (reduced hemolysis), hydrogen peroxide as negative control (increased hemolysis) and PDT and etoposide as standard anticancer molecules.

In conclusion, compounds **23** and **25** exhibited good cytotoxicity against colon cancer cell lines. Based on structure–activity relationship, it is evident that there is not much effect on cytotoxicity by shifting lactone to ring C and rings A and D are also not essential for cytotoxicity in these PDT analogues. From this lead, further study may be taken up to develop some better analogues as potent anticancer agents.

Experimental

General All the reference compounds (PDT, etoposide, taxol, *etc.*) and reagents for organic synthesis were purchased from Sigma and used without further purification. Melting points were determined on EZ-Melt automated MP apparatus (electrothermal), Stanford Research System, U.S.A. and were uncorrected. All the reactions were monitored on Merck aluminium thin layer chromatography (TLC, $UV_{254\,nm}$) plates with TLC visualization in UV (λ_{max} 254 and 365 nm) cabinet and also by spraying with a solution of 2% ceric sulphate in 10% aqueous sulfuric acid and charring at 100—110 °C. Column chromatography was carried out on silica gel (60—120 mesh, MERCK chemicals). NMR experiments were obtained on Bruker Avance-300 MHz instrument with tetramethylsilane (TMS) as internal standard. All the ^{1}H and necessary ^{13}C spectral data are reported. Electrospray ionization (ESI) mass spectra were recorded on Shimadzu LC-MS after dissolving the compounds in methanol. FT-IR spectra were recorded on Perkin-Elmer SpectrumBX. All the compounds were screened against four human cancer cell lines by MTT assay for cytotoxic evaluation. Compounds showing potent cytotoxicity were further evaluated for erythrocyte osmotic fragility test to determine their toxicities.

Synthesis of 3,4-Methylenedioxyphenyl Formate (5) 25 mg Piperonal **4** (0.166 mmol) was taken in 10 ml dry dichloromethane. The reaction mixture was cooled to 10 °C by ice-bath and 31 mg *m*-CPBA (0.18 mmol) was added to it. The reaction mixture was stirred for 2 h with cooling then stirred at room temperature for 6 h. On completion, the reaction mixture was washed with 5% sodium bicarbonate solution (3×25 ml) then washed with water. The organic layer was dried over anhydrous Na_2SO_4 and distilled off *in vacuo*. The residue thus obtained was purified through column chromatography over silica gel and eluted with hexane–ethyl acetate. The desired ester **5** was obtained as yellowish oil. Yield=48% mp=oil. ^{1}H-NMR ($CDCl_3$) δ: 5.89 (s, 2H, –O–CH_2–O–), 6.28 (d, 1H, 5-CH), 6.54 (s, 1H, 2-CH), 6.68 (d, 1H, 6-CH), 7.92 (s, 1H, O-CHO, formate). ESI *m/z*: negative ion mode; 165 $[M-H]^-$.

Synthesis of 3,4-Methylenedioxyphenyl Acetate (8) Procedure same as for **5**. Yield=47%, colour=light yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 2.26 (s, 3H, $OCOCH_3$), 5.9 (s, 2H, OCH_2O), 6.60 (d, 1H, aromatic), 6.52 (d, 1H, aromatic), 6.54 (s, 1H, aromatic).

Synthesis of 3,4-Methylenedioxyphenol (6) 534 mg Acetate ester **8** (29 mmol) was taken in 10 ml 5% aqueous–methanolic (1 : 1) KOH. The reaction mixture was heated at 50 °C for 2 h. On completion, the reaction mixture was acidified with 5% HCl, extracted with chloroform and washed with water. The organic layer was dried over anhydrous Na_2SO_4 and dried *in vacuo* to obtain a residue. This was purified through column chromatography over silica gel eluting with hexane–ethyl acetate. The desired phenol **6** was obtained as reddish brown solid. Yield=95%. mp=38 °C. ^{1}H-NMR ($CDCl_3$) δ: 5.89 (s, 2H, –OCH_2O–), 6.27 (dd, 1H, 6-CH, J=2.4, 8.4 Hz), 6.65 (d, 1H, 5-CH), 6.4 (s, 1H, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 31.06, 101.46, 107.18, 108.47, 142.06, 148.74, 151.2. ESI *m/z*: 138 $[M^+]$, 137 $[M^+-1]$.

General Procedure for Synthesis of Lactone Analogues of PDT (12, 13, 24—28) 277 mg Phenol **6** (1.1 mmol) and 125 mg 3,4,5-trimethoxycinnamic acid methyl ester **10** (0.9 mmol) were stirred in 2 ml trifluoroacetic acid at room temperature for 24 h. After completion, the reaction mixture was diluted with 20 ml water, washed with 5% $NaHCO_3$ solution (3×20 ml), extracted with chloroform. Organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to obtain a residue. This was purified through column chromatography over silica gel eluting with hexane–ethyl acetate. The desired product **12** was obtained as reddish brown

Table 2. MEF_{50} Values of Erythrocyte Osmotic Fragility of Different Compounds

S. No.	Condition	Mean erythrocyte fragility (50%)[a)]	Concentration (μg/ml)
1	Control	0.60	—
2	**14**	0.74	100
3	**19**	0.70	100
4	**22**	0.73	100
5	**23**	0.72	100
6	**25**	0.73	100
7	Podophyllotoxin	0.72	100
8	Etoposide	0.55	100
9	Curcumin	0.51	10
10	Hydrogen peroxide	0.77	10
11	Podophyllotoxin	0.71	10

a) Values are mean of three experiments in replicate.

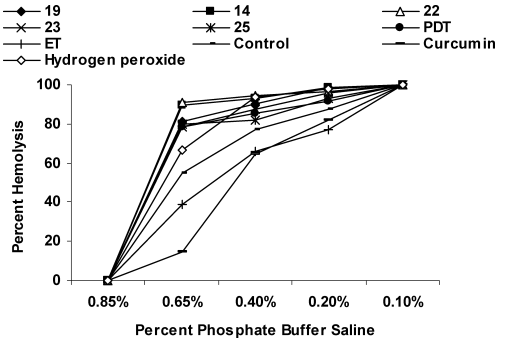

Fig. 2. Osmotic Haemolysis Curve of Erythrocytes

crystalline solid.

3,4-Dihydro-6,7-methylenedioxy-4-(3,4,5-trimethoxyphenyl)coumarin (**12**): Yield=34%. mp=188—190 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.96—3.02 (two pairs of dd, 2H, 3-CH_2, J=6, 8.1 Hz), 3.81 (s, 6H, 3′, 5′-OCH_3), 3.84 (s, 3H, 4′-OCH_3), 4.13—4.17 (t, 1H, 4-CH, J=6.75 Hz), 5.97 (s, 2H, –O–CH_2–O–), 6.35 (s, 2H, 2′, 6′-CH, aromatic), 6.44 (s, 1H, 5-CH, aromatic), 6.66 (s, 1H, 8-CH, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 37.37, 41.47, 56.73, 56.73, 61.08, 99.46, 99.46, 102.05, 105.59, 107.60, 118.32, 136.46, 138.57, 144.91, 146.67, 148.07, 154.24, 154.24, 167.63. IR (KBr) cm^{-1}: 1654, 1752. ESI m/z: 358 [M$^+$], 359 [M$^+$+1], 343 [M$^+$−CH_3].

3,4-Dihydro-7-methoxy-4-(3,4,5-trimethoxyphenyl)coumarin (**24**): Yield=16%. mp=102—104 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.94—3.01 (two pairs of dd, 2H, 3-CH_2, J=7.5, 9 Hz), 3.59 (s, 3H, 7-OCH_3), 3.77 (s, 3H, 4′-OCH_3), 3.80 (s, 6H, 3′, 5′-OCH_3), 4.74—4.80 (t, 1H, 4-CH, J=8 Hz), 6.31 (dd, 1H, 6-CH, aromatic, J=8.1, 2.4 Hz), 6.34 (d, 1H, 8-CH, aromatic, J=2.1 Hz), 6.45 (s, 2H, 2′, 6′ of aromatic), 6.90—6.93 (d, 1H, 5-CH, aromatic, J=8.1 Hz). ^{13}C-NMR ($CDCl_3$) δ: 40.19, 40.81, 51.75, 55.79, 56.61, 61.06, 99.97, 106.10, 106.21, 107.37, 124.41, 128.80, 137.31, 139.71, 153.42, 156.19, 158.40, 173.06. IR (KBr) cm^{-1}: 842, 1130, 1594, 1738. ESI m/z: 344 [M$^+$], 345 [M+1]$^+$.

3,4-Dihydro-6,7-methylenedioxy-4-(2-acetyloxy-3,5-dimethoxyphenyl)-coumarin (**14**): Yield=98%. mp=171—173 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (s, 3H, $OCOCH_3$), 2.96—3.04 (dd, 2H, 3-CH_2, J=6, 8.1 Hz), 3.77 (s, 6H, 3′, 5′-OCH_3), 4.15—4.17 (t, 1H, 4-CH, J=6.6 Hz), 5.97 (s, 2H, –O–CH_2–O–), 6.38 (s, 2H, 2′, 6′-CH, aromatic), 6.45 (s, 1H, 5-CH, aromatic), 6.66 (s, 1H, 8-CH, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 20.64, 37.28, 41.65, 56.64, 56.64, 99.50, 102.11, 104.78, 107.64, 117.97, 128.92, 139.34, 144.94, 146.66, 148.15, 153.15, 167.47, 168.76. IR (KBr, cm^{-1}): 1130, 1460, 1737, 1762. ESI m/z: 409 [M+Na]$^+$; negative mode, 385 [M−H]$^-$.

3,4-Dihydro-5,7-dimethoxy-4-(3,4,5-trimethoxyphenyl)coumarin (**25**): Yield=29%. mp=146—148 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.98—3.0 (br s, 2H, 3-CH_2,), 3.78 (s, 6H, 3′, 5′-OCH_3), 3.82 (s, 3H, 4′-OCH_3), 4.48—4.51 (br t, 1H, 4-CH), 6.29 (s, 1H, aromatic), 6.32 (s, 1H, aromatic), 6.34 (s, 2H, 2′, 6′-CH, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 35.19, 37.32, 55.89, 56.19, 56.19, 56.59, 60.98, 94.72, 95.69, 104.95, 106.76, 108.07, 137.70, 138.12, 153.54, 153.91, 157.94, 161.27, 167.80. IR (KBr) cm^{-1}: 834, 1128, 1626. ESI m/z: 397 [M+Na]$^+$; 413 [M+K]$^+$.

3,4-Dihydro-6,7-dimethoxy-4-(3,4,5-trimethoxyphenyl)coumarin (**26**): Yield=24%. mp=145—147 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.92—3.11 (two pairs of dd, 2H, 3-CH_2, J=7.2, 8.0 Hz), 3.77 (s, 3H, OCH_3), 3.81 (s, 6H, 3′, 5′-OCH_3), 3.84 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.19—4.23 (t, 1H, 4-CH, J=6.5 Hz), 6.35 (s, 2H, 2′, 6′-CH, aromatic), 6.51 (s, 1H, aromatic), 6.70 (s, 1H, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 37.85, 41.40, 56.63, 56.81, 56.81, 57.11, 61.01, 102.32, 105.93, 112.31, 116.93, 136.76, 146.28, 146.74, 150.34, 154.30, 167.51. IR (KBr) cm^{-1}: 1125, 1164, 1510, 1756. ESI m/z: 397.1 [M+Na]$^+$, 771.0 [2M+Na]$^+$, negative mode, 372.9 [M−H]$^-$.

3,4-Dihydro-7-methoxy-4-(3,4,5-trimethoxyphenyl)-2H-naphtho[2,3-*b*]pyran-2-one (**27**): Yield=16%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 3.10—3.23 (br d, 2H, 3-CH_2), 3.72 (s, 6H, 3′, 5′-OCH_3), 3.77 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 4.82—4.85 (br t, 1H, 4-CH), 6.31 (s, 2H, aromatic), 7.15—7.77 (m, 5H, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 22.94, 37.91, 55.71, 56.66, 56.66, 60.98, 105.18, 107.70, 112.19, 114.79, 118.25, 120.31, 124.89, 126.66, 128.94, 132.83, 136.66, 148.75, 154.29, 157.72, 167.30. IR (KBr, cm^{-1}): 784, 1260, 2927. ESI m/z: 394 [M]$^+$, 395 [M+1]$^+$.

3′,4′-Dihydro-4′-(3,4,5-trimethoxyphenyl)estra-1(10),2,4-trieno[3,2-*b*]pyran-2′,17-dione (**28**): Yield=58%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 0.89 (s, 3H, 18-CH_3), 1.39—2.25 (m, 15H, all the CH_2, CH of steroidal ring), 2.95—3.04 (br dd, 2H, 3-CH_2), 3.79 (s, 6H, 3′, 5′-OCH_3), 3.84 (s, 3H, OCH_3), 4.20—4.22 (br t, 1H, 4-CH), 6.33 (s, 1H, aromatic), 6.37 (s, 1H, aromatic), 6.87 (d, 1H, aromatic, J=3.3 Hz), 6.96 (s, 1H, aromatic). ESI m/z: 491 [M+H]$^+$, 513 [M+Na]$^+$.

General Procedure for Synthesis of Amides (16, 17, 20, 21) 120 mg 3,4,5-Trimethoxycinnamic acid **10** (1 mmol) was taken in 10 ml dry DMF. To this, 106 mg EDC (1.1 mmol), 75 mg HOBt (1.1 mmol), and 1 ml triethylamine were added and stirred at room temperature for 20 min. Later, 75 mg 3,4-methylenedioxyaniline (1.1 mmol) was added to the reaction mixture and further stirred for 6 h. On completion, 10 ml water was added and extracted with chloroform (3×20 ml). The organic layer was washed with water, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*. The residue thus obtained was purified through a silica gel column by eluting with hexane–ethyl acetate. The desired amide **16** was obtained as a creamy white solid.

(*E*)-*N*-(3,4-Methylenedioxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide (**16**): Yield=46%. mp=183—185 °C. ^{1}H-NMR ($CDCl_3$) δ: 3.89 (s, 9H, 3×OCH_3), 5.96 (s, 2H, –OCH_2–), 6.39—6.48 (d, 1H, =CH–CO–, J=15.3 Hz), 6.76 (s, 2H, aromatic), 6.78 (d, 1H, aromatic), 6.86 (d, 1H, aromatic), 7.36 (br s, 1H, aromatic), 7.62—7.67 (d, 1H, =CH, J=15.3 Hz). IR (KBr) cm^{-1}: 1127, 1656, 3266. ESI m/z: 358 [M+H]$^+$; 380 [M+Na]$^+$.

(*E*)-*N*-(3,4-Methylenedioxyphenyl)-3-(4-acetoxy-3,5-dimethoxyphenyl)-acrylamide (**17**): Yield=62%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 2.35 (s, 3H, $OCOCH_3$), 3.84 (s, 6H, 2×OCH_3), 5.9 (s, 2H, –OCH_2–), 6.41—6.46 (d, 1H, =CHCO, J=15 Hz), 6.75 (s, 2H, aromatic), 6.78 (d, 1H, aromatic), 6.87 (br d, 1H, aromatic), 7.61—7.66 (d, 1H, CH=C, J=15.6 Hz), 8.01 (s, 1H, aromatic). IR (KBr) cm^{-1}: 1199, 1499, 1560, 1753. ESI m/z: 408 [M+Na]$^+$, 793 [2M+Na]$^+$.

(*E*)-*N*-(3,4,5-Trimethoxyphenyl)-3-(3,4,5-trimethoxyphenyl)acrylamide (**20**): Yield=57%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 3.9 (s, 3H, OCH_3), 3.89 (s, 6H, 2×OCH_3), 3.84 (s, 9H, 3×OCH_3), 6.7 (s, 4H, aromatic), 6.50—6.56 (d, 1H, =CH–CO–, J=15.3 Hz), 7.63—7.68 (d, 1H, =CH, J=15.3 Hz). ESI m/z: 426 [M+Na]$^+$

(*E*)-*N*-(3,4,5-Trimethoxyphenyl)-3-(4-hydroxy-3,5-dimethoxyphenyl)acrylamide (**21**): Yield=55%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 2.31 (s, 3H, $OCOCH_3$), 3.87 (s, 6H, 2×OCH_3), 3.82 (s, 3H, OCH_3), 3.81 (s, 6H, 2×OCH_3), 5.8 (s, 1H, NH), 6.41—6.46 (d, 1H, =CH–C–, J=15.3 Hz), 6.71 (s, 4H, aromatic), 6.7 (s, 2H, aromatic). ESI m/z: 432 [M+H]$^+$.

General Procedure for Synthesis of Lactam Analogues of PDT (18, 19, 22, 23) 600 mg Amide **16** (1.6 mmol) was stirred in 2 ml trifluoroacetic acid at room temperature for 72 h. After completion, the reaction mixture was diluted with water, extracted with chloroform, and washed with 5% $NaHCO_3$ solution (3×20 ml). The organic layer was dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to obtain a residue. The residue thus obtained was purified through a silica gel column by eluting with hexane–ethyl acetate. The desired amide **18** was obtained as a creamy white solid.

3,4-Dihydro-6,7-methylenedioxy-4-(3,4,5-trimethoxyphenyl)quinolin-2(1*H*)-one (**18**): Yield=45%. mp=212—214 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.84—2.88 (two pairs of dd, 2H, 3-CH_2, J=3.3, 5.7 Hz), 3.81 (s, 6H, 3′, 5′-OCH_3), 3.84 (s, 3H, OCH_3), 4.08—4.13 (t, 1H, 4-CH, J=4.4 Hz), 5.92 (s, 2H, –O–CH_2–O–), 6.38 (s, 2H, 2′, 6′-CH, aromatic), 6.39 (s, 1H, aromatic), 6.41 (s, 1H, aromatic). ^{13}C-NMR ($CDCl_3$) δ: 38.94, 42.78, 56.68, 56.68, 61.10, 98.10, 101.63, 105.78, 108.74, 119.59, 129.15, 131.10, 131.76, 137.55, 144.09, 147.69, 154.05, 167.97, 170.88. IR (KBr) cm^{-1}: 1655, 1686, 1993. ESI m/z: 358 [M+H]$^+$; 380 [M+Na]$^+$; negative mode, 356 [M−H]$^-$.

3,4-Dihydro-6,7-methylenedioxy-4-(4-hydroxy-3,5-dimethoxyphenyl)-quinolin-2(1*H*)-one (**19**): Yield=34.5%. mp=262—264 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.83—2.87 (dd, 2H, 3-CH_2, J=4.2, 4.8 Hz), 3.86 (s, 6H, 3′, 5′-OCH_3), 4.07—4.12 (t, 1H, 4-CH, J=7.2 Hz), 5.92 (d, 2H, –O–CH_2–O–, J=1.8 Hz), 6.28 (s, 1H, aromatic), 6.38 (s, 1H, aromatic), 6.41 (s, 2H, 2′, 6′-CH, aromatic). IR (KBr) cm^{-1}: 1544, 1638, 1655. ESI m/z: 366 [M+Na]$^+$.

3,4-Dihydro-5,6,7-trimethoxy-4-(3,4,5-trimethoxyphenyl)quinolin-2(1*H*)-one (**22**): Yield=66%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 2.82—3.01 (two pairs of dd, 2H, 3-CH_2, J=7.2, 8.1 Hz), 3.77 (s, 3H, OCH_3), 3.80 (s, 6H, 3′, 5′-OCH_3), 3.84 (s, 3H, OCH_3), 3.86 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.52—4.54 (br d, 1H, 4-CH, J=5.1 Hz), 6.19 (s, 1H, aromatic), 6.37 (s, 2H, 2′, 6′-CH, aromatic), 8.06 (s,1H, exchangeable, amide NH). IR (KBr) cm^{-1}: 1655, 3399. ESI m/z: 426 [M+Na]$^+$; negative mode, 402 [M−H]$^-$.

3,4-Dihydro-5,6,7-trimethoxy-4-(4-hydroxy-3,5-dimethoxyphenyl)-quinolin-2(1*H*)-one (**23**): Yield=36.7%. Oil. ^{1}H-NMR ($CDCl_3$) δ: 2.85—2.94 (dd, 2H, 3-CH_2), 3.71(s, 3H, OCH_3), 3.79 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.51 (m, 1H, 4CH), 5.38 (br s, 1H exchangeable, NH), 6.36 (s, 1H, aromatic), 6.80 (s, 2H, aromatic). ESI mass (MeOH): 390 [M+H]$^+$; 412 [M+Na]$^+$.

Cell Assays Human cancer cell lines were American type of cell culture collection (ATCC) obtained from NCCS Pune, India. Cells were cultured in DMEM with HEPES-25 mM, 0.22% $NaHCO_3$, and 10% FBS.

In vitro cytotoxicity testing was performed as per reported method.[14] 2×10^3 cells/well were incubated in 5% CO_2 incubator for 24 h to enable them to adhere properly to the 96 well polystyrene microplate (Grenier, Germany). Test compound dissolved in dimethyl sulfoxide (DMSO, Merck, Germany), in at least five concentrations, was added into the wells and left for 4 h. After the incubation, the compound plus media was replaced with fresh media and the cells were incubated in the CO_2 incubator at 37 °C for another 48 h. The concentration of DMSO was always kept below 1.25%, which was found to be non-toxic to cells. Then, 10 μl MTT was added to each well and plates were incubated at 37 °C for 4 h. 100 μl DMSO was added to all wells and mixed thoroughly to dissolve the dark blue crystals. The plates were read on SpectraMax 190 Microplate reader (Molecular Devices Inc., U.S.A.) at 570 nm within 1 h of DMSO addition. The experiment

was done in triplicate and the inhibitory concentration (IC) values were calculated. IC_{50} is the concentration μg/ml required for 50% inhibition of cell growth as compared with that of untreated control.

The toxicity evaluation with human erythrocytes was done as per reported method.[15] Results are expressed in terms of mean erythrocyte fragility (MEF_{50}), which is the level of hemolysis of the erythrocytes at 50% saline concentrations (Table 2). The MEF_{50} values at standard pH and temperature were then obtained from the curve.

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