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11*H*-Isoquino[4,3-*c*]cinnolin-12-ones: novel anticancer agents with potent topoisomerase I-targeting activity and cytotoxicity

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Abstract—Recent studies have identified 2,3-dimethoxy-8,9-methylenedioxy-11-[(2-dimethylamino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (**1a**) as a novel topoisomerase I-targeting agent with potent cytotoxic activity. The effect of varied substituents at the 11-position of 2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-ones on topoisomerase I-targeting activity and cytotoxicity was evaluated. Potent TOP1-targeting activity was observed when the 11-position was substituted with either a 2-(*N*,*N*-dimethylamino)ethyl, a 2-(*N*,*N*-diethylamino)ethyl, a *n*-butyl, or a 2-(pyrrolidin-1-yl)ethyl group. The addition of a β-methyl group to **1a** provided an analogue with dramatically reduced TOP1-targeting activity and cytotoxicity. Analogues of **1a** wherein the 2-(*N*,*N*-dimethylamino)ethyl group was replaced with a (2-tetrahydrofuranyl)methyl, a 2-(piperidin-1-yl)ethyl, or a 2-(4-methylpiperazin-1-yl)ethyl substituent exhibited decreased activity as TOP1-targeting agents. Replacement of the dimethoxy groups of **1a** with hydrogen atoms resulted in an analogue with significantly decreased TOP1-targeting activity and cytotoxicity. Removal of both the vicinal dimethoxyl groups and the methylenedioxy moiety resulted in a complete loss of TOP1-targeting activity. The presence of a 9-nitro substituent in place of the 8,9-methylenedioxy group of **1a** resulted in a decrease in relative TOP1-targeting activity and cytotoxicity. Compounds **1a** and the 11-*n*-butyl analogue **1d** were evaluated for antitumor activity in the human tumor xenograft model using athymic nude mice. The non-estrogen responsive breast tumor cell line MDA-MB-435 was used in these assays. At dose levels that approached its maximum tolerated dose, **1a** proved to be effective in inhibiting tumor growth in vivo when administered orally or by ip injection.

1. Introduction

Topoisomerases are nuclear enzymes that regulate the topology of DNA and are critical for replication and transcription. There are two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2), which are distinguished based upon differences in their initial mechanisms wherein a single or double-stranded DNA break is implicated.[1–4] Topoisomerase-targeting agents stabilize the cleavable complex formed between the enzyme and DNA resulting in the formation of double-strand breaks. Topoisomerase-targeting agents are acknowledged as effective in the treatment of cancer. The drug-induced stabilization of the enzyme–DNA cleavable complex effectively converts these nuclear enzymes into cellular poisons.

Camptothecin was the first compound identified as a TOP1-targeting agent.[5] From the extensive studies on camptothecin and its structurally related analogues, two clinical agents, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®), have been developed. As in camptothecin, these drugs have incorporated within their structures a γ-lactone that is susceptible to hydrolysis. Ring-opening of this lactone results in the formation of an inactive derivative with high affinity for human serum albumin.[6–8] The instability of this lactone and the observation that both topotecan and irinotecan are substrates for efflux transporters associated with

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resistance have prompted further studies on the development of novel TOP1-targeting agents.[9–12]

There have been several reports on novel non-camptothecin TOP1-targeting agents. These include derivatives of bi- and terbenzimidazoles,[13,14] benz[*a*]anthracenes,[15] benzo[*c*]phenanthridine and protoberberine alkaloids,[16,17] indolocarbazoles,[18] the fungal metabolites bulgarein[19] and saintopin,[20] and several indenoisoquinolines,[21] benzophenazines[22] and benzo[*i*]phenanthridines.[23,24] These studies were performed in an effort to develop TOP1-targeting agents with the potential for improved efficacy.

Studies in our laboratory have recently demonstrated that 2,3 - dimethoxy - 8,9 - methylenedioxydibenzo[*c*,*h*]cinnoline,[25] 5*H*-dibenzo[*c*,*h*][1,6]naphthyridin-6-ones[26,27] and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones[26] can exhibit potent TOP1-targeting activity and pronounced cytotoxicity (Chart 1).

In the present study, the synthesis and pharmacological evaluation of several 11-substituted 2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-ones and variously substituted 11-(2-dimethylamino)ethyl-11*H*-isoquino[4,3-*c*]cinnolin-12-ones, as outlined in Fig. 1, were performed. The results of these studies are detailed.

2. Chemistry

A series of 11-substituted 2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-ones were synthesized as outlined in Scheme 1. Compound **6** was prepared from 6,7-methylenedioxy-4-cinnolone by treatment with a mixture of PCl_5 and $POCl_3$. The desired 4-alkylaminocinnolines, **7a–h**, were then prepared by reaction of the appropriately substituted primary alkylamine with **6**.

Conversion of 2-iodo-4,5-dimethoxybenzoic acid to its acid chloride and subsequent treatment with the appropriate 4-amino-6,7-methylenedioxycinnoline and TEA in anhydrous methylene chloride provided the amides **8a–h**. For those analogues that did not possess a dialkylaminoethyl group, relatively poor yields of the desired amides were obtained. In the case of these various 4-aminocinnoline derivatives, competitive acylation at the N1 position of the cinnoline moiety significantly lowered the yield. This result is consistent with previous studies on the reactivity of 4-aminocinnolines,[28] and explains the poor yields obtained in the acylation of **8d** and **8e** (19 and 34%, respectively). In the case of 4-[(2-dialkylaminoethyl)amino]cinnolines, however, acylation of the 4-amino group occurs exclusively and the yield obtained for **8a–c** ranged from 75 to 87%.

These results suggest that the strong tendency for 4-dialkylaminoethylaminocinnolines to form an intramolecular hydrogen bond through a cyclic five-membered structure, which has been documented in other studies,[29] promotes the desired acylation of the 4-amino substituent. The tendency to form such intramolecular hydrogen bonds would increase the electron density of the 4-amino group, thereby increasing its nucleophilicity.

Intramolecular cyclization was performed with the *o*-iodobenzamides, **8a–h**, using the Heck reaction, to provide the 11-substituted 11*H*-isoquino[4,3-*c*]cinnolin-12-ones, **1a–h**. The Heck reaction conditions that were employed in this study were those that proved optimal for formation of benzo[*c*]phenanthridine derivatives.[30]

The preparation of 11-(2-dimethylaminoethyl)-11*H*-isoquino[4,3-*c*]cinnolin-12-ones **2–5** was accomplished using the approach outlined in Scheme 2. 4-Chlorocinnoline **9** was prepared as described in the literature and refluxed with *N*,*N*-dimethylethylenediamine to provide **11**. Chlorination of 6-nitro-4-cinnolone with $POCl_3$ (either with or without PCl_5) provides poor yields of 4-chloro-6-nitrocinnoline, due to the replacement of the nitro group by chloride. Under different conditions, using thionyl chloride and a catalytic amount of PCl_5 in chlorobenzene, 4-chloro-6-nitrocinnoine was prepared as previously described.[31] As 4-chloro-6-nitrocinnoline is reportedly unstable, it was converted in situ to 4-phenoxy-6-nitrocinnoline **10**,[31] which could be isolated and served as a useful intermediate for the preparation of **12**.

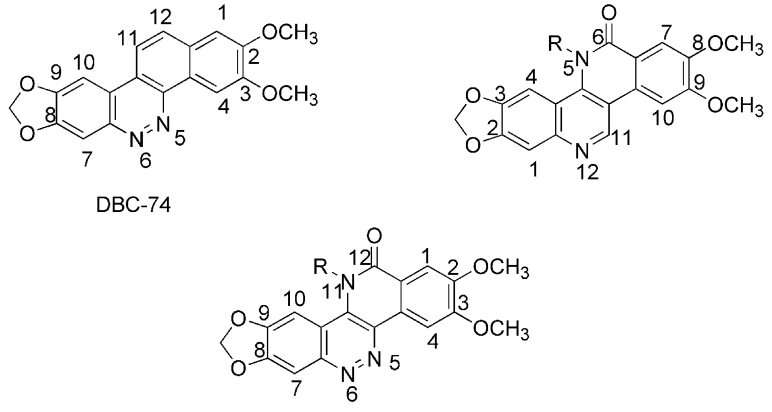
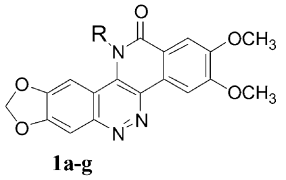
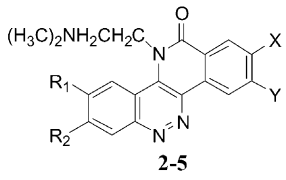


Chart 1. Structure and numbering of 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (DBC-74), 5*H*-dibenzo[*c*,*h*][1,6]-naphthyridin-6-ones and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones.



1a-g

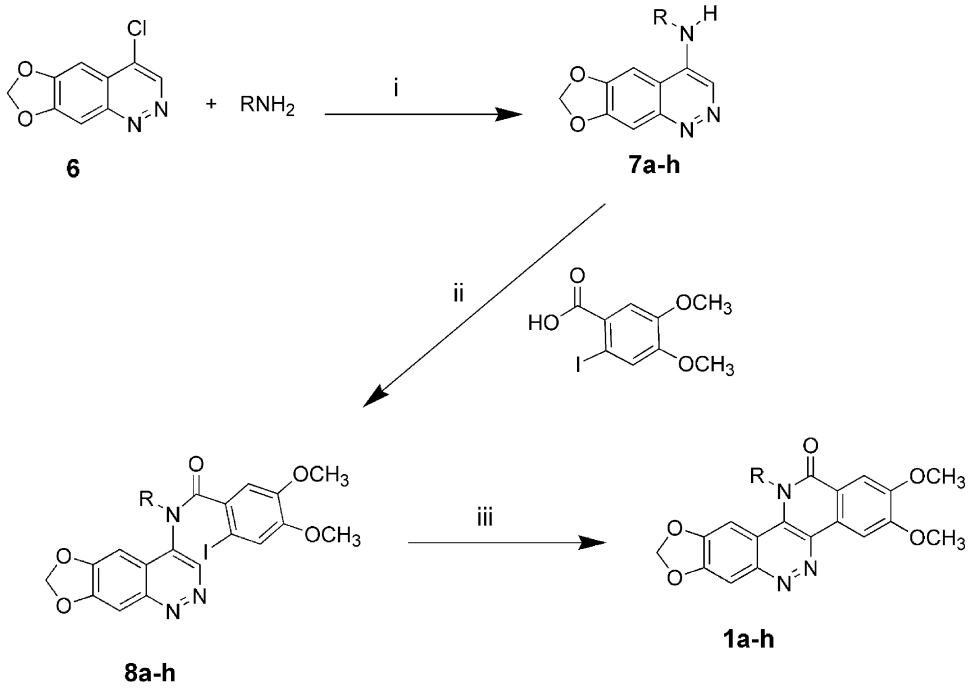


2-5

1a R = $CH_2CH_2N(CH_3)_2$
1b R = $CH_2CH_2N(CH_2CH_3)_2$
1c R = $CHCH_3CH_2N(CH_3)_2$
1d R = $CH_2CH_2CH_2CH_3$
1e R = $CH_2(CHOCH_2CH_2CH_2)$
1f R = $CH_2CH_2N(CH_2CH_2CH_2CH_2)$
1g R = $CH_2CH_2N(CH_2CH_2CH_2CH_2CH_2)$
1h R = $CH_2CH_2N(CH_2CH_2)_2NCH_3$

2 X = H; Y = H; R_1,R_2 = -OCH_2O-
3 X = H; Y = H; R_1 = H; R_2 = H
4 X = OCH_3; Y = OCH_3; R_1 = H; R_2 = H
5 X = OCH_3; Y = OCH_3; R_1 = NO_2; R_2 = H

Figure 1. The 11-substituted 11*H*-isoquino[4,3-*c*]cinnolin-12-ones synthesized and evaluated in this study.



Scheme 1. (i) Reflux or $\leq$135 °C; (ii) $(COCl)_2$, CH_2Cl_2, then TEA, CH_2Cl_2; (iii) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, DMF.

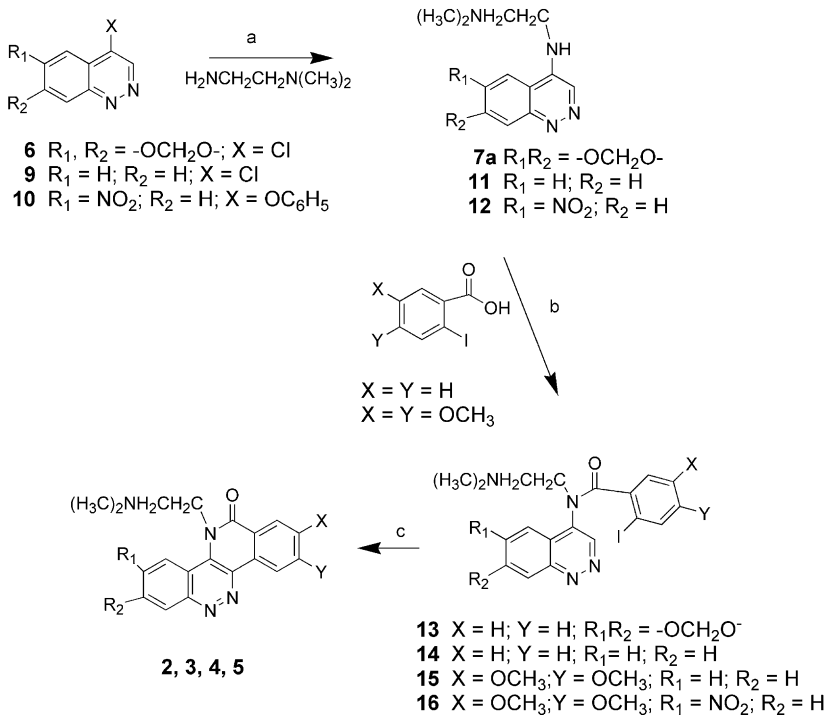
Treatment of **7a** and **11** with the acid chloride derived from 2-iodobenzoic acid provided the *o*-iodobenzamides **13** and **14**, respectively. Reaction of **11** and **12** with the acid chloride formed from 2-iodo-4,5-dimethoxybenzoic acid yielded the amides, **15** and **16**. Using Heck intramolecular cyclization conditions, **13**–**16** were converted to **2**–**5**.

3. Pharmacology

3.1. In vitro studies

The 11-substituted 11*H*-isoquino[4,3-*c*]cinnolin-12-ones synthesized in this study were evaluated for their relative TOP1-targeting activity and for cytotoxicity. These data are provided in Table 1. Representative gels illustrating the potential of some of these compounds at various concentrations to stabilize the cleavable complex formed in the presence of TOP1 and DNA are illustrated in Figures 2 and 3.

Compounds **1a**–**h** represent several 11-substituted derivatives of 2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-one. The presence of either a 2-(*N*,*N*-dimethylamino)ethyl, 2-(*N*,*N*-diethylamino)ethyl, a *n*-butyl, or 2-(pyrrolidin-1-yl)ethyl substituent at the 11-position is associated with potent TOP1-targeting activity. All four of these analogues, **1a**, **1b**, **1d**, and **1f** had IC_{50} values <5 nM when evaluated for cytotoxicity in the human lymphoblast cell line, RPMI8402. Each of these compounds also exhibited significant cross resistance in the camptothecin-resistant variant cell line, CPT-K5. Addition of a β-methyl substituent to the 11-(2-aminoethyl) side chain as in **1c** adversely affects TOP1-targeting activity and cytotoxicity. Compound **1c** is more than 300-fold less active as a TOP1-targeting agent and is over two orders of magnitude less cytotoxic than **1a**.



Scheme 2. (i) Reflux or $\leq 135\,^{\circ}C$; (ii) $(COCl)_2$, CH_2Cl_2, then TEA, CH_2Cl_2; (iii) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, DMF.

The 2-(tetrahydrofuranyl)methyl derivative **1e** is less active as a TOP1-targeting agent than the 2-(pyrrolidin-1-yl)ethyl analogue **1f** and less cytotoxic than **1f** to RPMI8402 and P388 cells. Cross-resistance was observed with **1e** to the camptothecin-resistant variants, CPT-K5 and P388/CPT45, respectively.[32,33]

Comparison of the relative TOP1-targeting activity of **1f** with either **1g** or **1h** suggests that additional steric constraints, other than substitution at the β-position of the ethyl side chain, at the 11-position may influence TOP1-targeting activity. While **1g** is 10-fold less potent than **1f** as a TOP1-targeting agent, it exhibits similar cytotoxic activity. Based upon the cross-resistance observed in both CPT-K5 and P388/CPT45 cells with **1g**, it appears that its cytotoxicity is largely mediated by interaction with TOP1. Compound **1h** is two orders of magnitude less active as a TOP1-targeting agent than **1f**, and is also significantly less cytotoxic in RPMI8402 or P388 cells (75–80 nM vs 3.0 nM). In addition to increased steric bulk at the 11-position, **1h** also has an additional tertiary amine that may be associated with its decreased activity.

The effect of removal of the dimethoxyl groups from **1a** was investigated by evaluating the relative activity of **2**. For compound **2**, we observed over a thirtyfold decrease in TOP1-targeting activity and over a hundredfold difference in cytotoxicity in RPMI8402 relative to **1a**. Replacement of the methylenedioxy moiety of **1a** with hydrogen, as in **4**, results in a slight reduction in

Table 1. Relative TOP1-targeting activity and cytotoxicity of 11-substituted 11*H*-isoquino[4,3-*c*]cinnolin-12-ones

Compd	TOP1-mediated DNA cleavage	Cytotoxicity IC_{50} (μM)			
		RPMI8402	CPT-K5	P388	P388/CPT45
1a	0.3	0.002	0.74	0.002	0.23
1b	0.3	0.004	0.51	0.002	0.28
1c	100	0.26	3.6	0.11	0.56
1d	0.5	0.004	10	0.004	1.2
1e	5.0	0.01	>10	0.02	1.8
1f	0.2	0.003	0.6	0.003	0.26
1g	2.0	0.003	1.1	0.003	0.27
1h	20	0.08	8.0	0.08	1.1
2	10	0.24	2.5	0.09	0.11
3	>1000	3.3	5.0	0.12	0.18
4	0.8	0.03	1.4	0.032	0.18
5	5.0	0.03	0.67	0.033	0.55
DBC-74	8.0	0.05	>10	0.033	>10
CPT	0.5	0.005	61	0.009	>10
CPT-11	25	0.57	>100	2.3	>100
Topotecan	1.0	0.012	>50	0.04	>10
VM-26	>1000	0.22	0.28	0.006	0.001

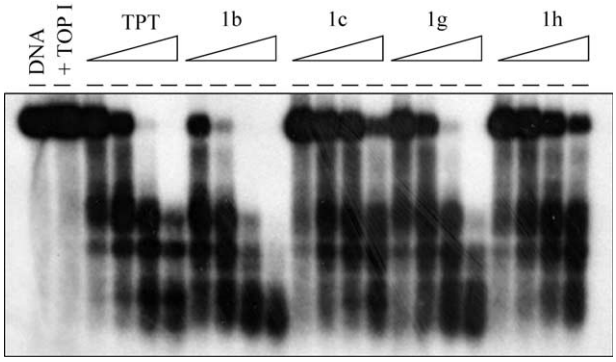


Figure 2. Stimulation of enzyme-mediated DNA cleavage by topotecan (TPT), **1b**, **1c**, **1g**, and **1h** using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

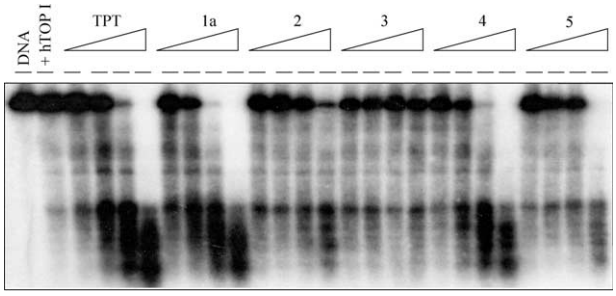


Figure 3. Stimulation of enzyme-mediated DNA cleavage by topotecan (TPT), **1a**, **2**, **3**, **4**, and **5** using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

TOP1-targeting activity, and a decrease in cytotoxicity that is greater than an order of magnitude. Replacement of not only the vicinal methoxyl groups, but also the methylenedioxy substituent of **1a** with hydrogen atoms, as in **3**, results in a loss of TOP1-targeting activity and significantly decreased cytotoxic activity. Studies in our laboratory have demonstrated that a nitro substituent at the 3 position of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]-naphthyridin-6-one allows for retention of significant TOP1-targeting activity and cytotoxicity (unpublished results). The analogous nitro analogue of 2,3-dimethoxy-11*H*-isoquino[4,3-*c*]cinnolin-12-one, **5**, was prepared in the present study to examine its activity as a TOP1-targeting agent and its relative cytotoxic activity. A decrease in TOP1-targeting activity relative to **1a** was observed for **5** similar in magnitude to that observed for **1e**. Compound **5**, however, did exhibit significant cytotoxicity in RMI8402 and P388 cells as well as significant cross-resistance in their camptothecin-resistant variants.

3.2. In vivo assays

Compound **1a** and **1d** as well as 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (Chart 1, DBC-74) were selected for evaluation in the human tumor xenograft athymic nude mouse model. Using the human breast tumor cell line, MDA-MB-435, tumor cells were implanted into athymic nude mice. Two separate bioassays were performed. Compound **1a**, as detailed in the Experimental, was administered by both gavage (po) and intraperitoneal injection (ip) in assay at doses that approached its maximum tolerated dose (MTD). In assay B, **1a** was administered po. As **1d** and 2,3-dimethoxy-8,9-dimethoxydibenzo[*c*,*h*]cinnoline (DBC-74) lack sufficient solubility in vehicles suitable for ip administration, these compounds were given by po administration as outlined for assay B. The limited bioavailability of **1d** and 2,3-dimethoxy-8,9-methylenedioxydibenzo[*c*,*h*]cinnoline (DBC-74, Chart 1) precluded establishing meaningful MTD levels for these compounds. Table 2 summarizes the results obtained from these assays. Despite escalation of dose, there was no effect on tumor growth observed after more than two weeks of treatment in mice treated with either **1d** or DBC-74. Mice in these experimental groups were terminated on day 16. The total dose reported in Table 2 reflects the total dose administered to each experimental group for the duration of the study.

The data from both assay A and assay B suggest that **1a** does exert antitumor activity in vivo. While **1a** appears to be quite potent, its dose-limiting toxic effects appear to limit its efficacy in these bioassays. Relative to CPT-11, this analogue as substantially less efficacious when administered ip. The lack of activity observed with **1d** and DBC-74 are in all likelihood associated with their poor solubility and limited bioavailability. While both of these compounds are cytotoxic in vitro, their limited bioavailability most likely excludes detectable antitumor activity in vivo. Despite evidence to suggest that **1a** exhibits somewhat greater potency in vitro, as noted in an earlier communication, it appears to be less efficacious in vivo than similarly substituted dibenzo[*c*,*h*]-naphthyridin-6-ones. Differences in the metabolic fate of members within each of these families of novel TOP1-targeting agents are likely responsible for this difference between in vitro and in vivo results.

4. Experimental

Melting points were determined with either a Thomas-Hoover Unimelt or Meltemp capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32-63 μm, (ICN Biomedicals, Eschwege, Germany) using the solvent systems indicated. Proton (^{1}H NMR) and carbon (^{13}C NMR) nuclear magnetic resonance were recorded on a Varian Gemini-200 Fourier Transform spectrometer. Infrared spectral data were obtained using a Thermo-Nicolet Avatar 360 Fourier transform spectrophotometer and are reported in cm^{-1}. NMR spectra (200 MHz ^{1}H and 50 MHz ^{13}C) were recorded in the deuterated solvent indicated with chemical shifts reported in δ units downfield from tetramethylsilane (TMS). Coupling constants are reported in hertz (Hz). Mass spectra were obtained from Washington University Resource for Biomedical and Bio-organic Mass Spectrometry within the Department of Chemistry at Washington University, St. Louis, MO, USA.

Table 2. Antitumor activity observed in athymic nude mice with the human tumor xenograft MDA-MB-435

Compd	Assay[a]	# of mice	Route	Average tumor volume (mm^3)				Total dose (mg/kg)/mouse
				Day 7	Day 16	Day 21	Day 31	
1a	A	7	po[b]	159	148	99	62	0.78
1a	A	7	ip[b]	123	84	65	40	0.72
Vehicle	A	7	ip[c]	133	152	188	298	
CPT-11	A	7	ip[d]	99	98	40	14	17.38
1a	B	7	po[e]	139	194	190	76	0.29
1d	B	7	po[f]	206	345	—	—	4.34
DBC-74	B	7	po[f]	209	267	—	—	4.76
Vehicle	B	7	ip[c]	199	277	356	472	
CPT-11	B	7	ip[d]	119	65	30	10	13.89

[a] Two separate assays were performed. Both assay A and B used female NCR/NU NU mice.
[b] Initial dose was 2.0 mg/kg qd×4/week. Administration was adjusted to approximately 3×week in view of weight loss. Dosing was withheld from some mice with notable weight loss.
[c] Vehicle consisted of 0.1% citrate in H_2O.
[d] Administered at a dose of 20 mg/kg×5/week.
[e] Initial dose was 1.0 mg/kg qd×3/week. Dosing was withheld from some mice with notable weight loss.
[f] Administered initially at 10 mg/kg qd×3/week, increased to 10 mg/kg qd×7/week. Dose administered was limited by the solubility of compound in 30% Cremophor EL adjusted to pH 3.0.

4.1. General procedure for the preparation of 11-substituted 11*H*-isoquino[4,3-*c*]cinnolin-12-ones via cyclization of *o*-iodobenzamides

A mixture of the 4-amino-6,7-methylenedioxycinnoline *o*-iodobenzamide derivative (1.0 mmol equiv), $Pd(OAc)_2$ (0.2 mmol equiv), P(*o*-tolyl)$_3$ (0.4 mmol equiv), and Ag_2CO_3 (2.0 mmol equiv) was heated to reflux in DMF (30 mL per mmol equiv) with stirring. The reaction mixture was allowed to cool to room temperature, diluted with $CHCl_3$, and filtered through Celite. The sicciate was extensively washed with 10% CH_3OH in $CHCl_3$. The filtrate was concentrated in vacuo and the residue chromatographed on silica gel using chloroform:methanol.

4.1.1. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-dimethylamino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1a). Prepared from **8a** (220 mg, 0.40 mmol); (36% yield); reaction time 25 min; mp 288–289 °C (dec.); IR ($CHCl_3$) 1653; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 3.04 (t, 2H, J = 7.2), 4.08 (s, 3H), 4.17 (s, 3H), 4.64 (t, 2H, J = 7.2), 6.25 (s, 2H), 7.81 (s, 1H), 7.84 (s, 1H), 8.07 (s, 1H), 8.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 47.4, 56.4, 56.7, 57.7, 99.4, 102.8, 104.3, 106.6, 107.9, 113.7, 119.6, 129.1, 131.0, 134.4, 149.4, 150.2, 151.5, 154.4, 163.1; HRMS calcd for $C_{22}H_{22}O_5N_4H$: 423.1668; found: 423.1653.

4.1.2. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-diethylamino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1b). Prepared from **8b** (578 mg, 1.0 mmol); (18% yield); reaction time 25 min; mp 245–247 °C (dec.); IR ($CHCl_3$) 1652; ^{1}H NMR ($CDCl_3$) δ 1.08 (t, 6H, J = 7.0), 2.67 (q, 4H, J = 7.0), 3.14 (t, 2H, J = 7.1), 4.08 (s, 3H), 4.17 (s, 3H), 4.64 (t, 2H, J = 7.1), 6.25 (s, 2H), 7.80 (s, 1H), 7.84 (s, 1H), 8.18 (s, 1H), 8.63 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.8, 47.7, 48.0, 51.5, 56.4, 56.6, 99.7, 102.7, 104.3, 106.4, 108.0, 113.7, 119.7, 129.1, 131.1, 134.4, 149.4, 150.3, 151.2, 151.5, 154.4, 163.2; HRMS calcd for $C_{24}H_{26}O_5N_4H$: 451.1952; found: 451.1960.

4.1.3. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-dimethylamino)-1-methylethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1c). Prepared from **8c** (100 mg, 0.18 mmol); (28% yield); reaction time 2 h; mp 235–236 °C; IR (KBr) 1659: ^{1}H NMR ($CDCl_3$) δ 1.93 (d, 3H, J = 8.2), 1.97 (s, 3H), 2.74 (dd, 1H, J = 5.8,13.6), 3.27 (dd, 1H, J = 7.4,12.8), 4.07 (s, 3H), 4.15 (s, 3H), 4.80 (m, 1H), 6.24 (s,2H), 7.74 (s,1H), 7.81 (s,1H), 8.57 (s,1H); ^{13}C NMR ($CDCl_3$) δ 19.4, 45.6, 56.3, 58.6, 63.0, 99.0, 102.6, 104.1, 106.2, 107.9, 114.2, 120.8, 125.6, 128.6, 131.0, 132.5, 132.8, 135.1, 149.2, 150.3, 150.6, 151.3, 154.2, 164.0; HRMS calcd for $C_{23}H_{24}N_4O_5H$ 436.1826; found 436.1832.

4.1.4. 2,3-Dimethoxy-8,9-methylenedioxy-11-(*n*-butyl)-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1d). Prepared from **8d** (123 mg, 0.2 mmol); (27% yield); reaction time 90 min; mp 299 °C; IR (KBr) 1654; ^{1}H NMR ($CDCl_3$) δ 1.06 (t, 3H, J = 7.4), 1.56 (m, 2H), 2.13 (m, 2H), 4.09 (s, 3H), 4.17 (s, 3H), 4.49 (m, 2H), 6.26 (s, 2H), 7.62 (s, 1H), 7.85 (s, 1H), 7.87 (s, 1H), 8.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.8, 20.2, 31.1, 48.6, 56.3, 56.6, 98.8,102.7, 104.2, 106.5, 107.9, 119.7, 149.1, 150.1, 150.9, 151.4, 153.6, 154.2, 162.9; HRMS calcd for $C_{22}H_{21}N_3O_5H$: 408.1559; found 408.1543.

4.1.5. 2,3-Dimethoxy-8,9-methylenedioxy-11-(2-tetrahydrofuranyl)methyl-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1e). Prepared from **8e** (140 mg, 0.25 mmol); (22% yield); reaction time 45 min; mp 300–303 °C (dec.); IR ($CHCl_3$) 1653; ^{1}H NMR ($CDCl_3$) δ 1.79 (m, 1H), 2.00 (m, 2H), 2.25 (m, 1H), 3.87 (m, 2H), 4.09 (s, 3H), 4.18 (s, 3H), 4.65 (m, 3H), 6.25 (s, 2H), 7.80 (s, 1H), 7.84 (s, 1H), 8.32 (s, 1H), 8.63 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.7, 30.8, 53.0, 56.4, 56.7, 68.4, 77.8, 100.0, 102.7, 104.3, 106.3, 108.0, 114.1, 119.7, 129.1, 131.4, 134.5, 149.5, 150.2, 150.8, 151.4, 154.4, 163.7; HRMS calcd for $C_{23}H_{21}O_6N_3$: 435.1430; found: 435.1427.

4.1.6. 2,3-Dimethoxy-8,9-methylenedioxy-11-[2-(pyrrolidin-1-yl)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1f). Prepared from **8f** (150 mg, 0.2 mmol); (24% yield); reaction time 30 min; mp 229 °C; IR (KBr) 1644; ^{1}H NMR ($CDCl_3$) δ 1.83 (m, 4H), 2.71 (m, 4H), 3.23 (t, 2H, J = 7.0), 4.06 (s, 3H), 4.61 (s, 3H), 4.63 (t, 2H, J = 7.0),

6.23 (s, 2H), 7.74 (s, 1H), 7.80 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.7, 54.0, 54.2, 56.3, 56.6, 99.4, 102.7, 104.2, 106.3, 107.7, 113.5, 119.4, 129.0, 134.1, 140.2, 150.2, 151.4, 154.3, 154.3, 163.0; HRMS calcd for $C_{24}H_{24}N_4O_5H$: 449.1825; found: 449.1822.

4.1.7. 2,3-Dimethoxy-8,9-methylenedioxy-11-[2-(piperidin-1-yl)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1g). Prepared from **8g** (295 mg, 0.5 mmol); (32.4% yield); reaction time 30 min; mp 294–295 °C; IR (KBr) 1662; ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 6H), 2.51 (s, 4H), 3.02 (t, 2H, $J=6.6$), 4.08 (s, 3H), 4.17 (s, 3H), 4.64 (t, 2H, $J=6.6$), 6.26 (s, 2H), 7.81 (s,1H), 7.85 (s, 1H), 8.36 (s, 1H), 8.65 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.3, 26.0, 47.5, 55.0, 56.3, 56.6, 57.4, 99.9, 102.7, 104.2, 106.3, 107.9, 113.7, 119.6, 129.0, 131.1, 134.3, 149.3, 150.2, 151.1, 151.4, 154.3, 163.1; HRMS calcd for $C_{25}H_{26}N_4O_5H$: 463.1981; found: 463.1986.

4.1.8. 2,3-Dimethoxy-8,9-methylenedioxy-11-[2-(4-methylpiperazin-1-yl)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (1h). Prepared from **8h** (726 mg, 1.2 mmol); (23% yield); reaction time 30 min; mp 292–295 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 2.29 (s, 3H), 2.40 (m, 4H), 2.61 (m, 4H), 3.04 (t, 2H, $J=6.6$), 4.08 (s, 3H), 4.17 (s, 3H), 4.64 (t, 2H, $J=6.6$), 6.25 (s, 2H), 7.78 (s, 1H), 7.82 (s, 1H), 8.16 (s, 1H), 8.61 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 46.1, 47.2, 53.5, 55.1, 56.4, 56.5, 56.7, 99.6, 102.7, 104.3, 106.4, 108.0, 113.7, 119.6, 129.0, 131.1, 134.4, 149.3, 150.2, 151.2, 151.5, 154.4, 163.2; IR ($CHCl_3$) 1653; HRMS calcd for $C_{25}H_{27}N_5O_5H$: 478.2090; found: 478.2088.

4.1.9. 8,9-Methylenedioxy-11-[2-(*N*,*N*-dimethylamino)-ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (2). Prepared from **13** (343 mg, 0.7 mmol); (22% yield); reaction time 25 min; mp 229–230 °C; IR ($CHCl_3$) 1663; ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 6H), 2.92 (t, 2H, $J=6.6$), 4.58 (t, 2H, $J=6.6$), 6.38 (s, 2H), 7.79 (m, 2H), 7.99 (s, 1H), 8.00 (m, 1H), 8.34 (d, 1H, $J=8.4$), 9.04 (d, 1H, $J=8.0$); ^{13}C NMR (DMSO-d_6) δ 45.1, 47.8, 56.9, 99.9, 104.1, 105.9, 113.9, 123.7, 125.8, 127.1, 128.4, 130.7, 134.1, 134.6, 139.6, 150.1, 151.5, 151.9, 163.5; HRMS calcd for $C_{20}H_{18}O_3N_4H$: 363.1457; found: 363.1453.

4.1.10. 11-[(2-Dimethylamino)ethyl]-11*H*-isoquino[4,3-*c*]-cinnolin-12-one (3). Prepared from **14** (140 mg, 0.4 mmol); (15% yield); reaction time 30 min; mp 179 °C; IR (KBr) 1664; ^{1}H NMR ($CDCl_3$) δ 2.41 (s, 6H), 3.06 (t, 2H, $J=7.4$), 4.76 (t, 2H, $J=6.8$), 7.72–8.00 (m, 4H), 8.50 (d, 1H, $J=8.0$), 8.65–8.70 (m, 2H), 9.30 (d, 1H, $J=7.8$); ^{13}C NMR ($CDCl_3$) δ 45.9, 47.4, 57.3, 123.6, 124.0, 125.5, 127.9, 130.0, 130.5, 131.3, 133.8, 163.4; HRMS calcd for $C_{19}H_{18}N_4OH$: 319.1551; found 319.1560.

4.1.11. 11-[(2-Dimethylamino)ethyl]-2,3-dimethoxy-11*H*-isoquino[4,3-*c*]cinnolin-12-one (4). Prepared from **15** (150 mg, 0.3 mmol); (40% yield); reaction time 25 min; mp 218–219 °C; IR ($CHCl_3$) 1655; ^{1}H NMR ($CDCl_3$) δ 2.42 (s, 6H), 3.06 (t, 2H, $J=7.5$), 4.09 (s, 3H), 4.19 (s, 3H), 4.76 (t, 2H, $J=7.5$), 7.85 (m, 3H), 8.67 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 45.9, 47.3, 56.4, 56.7, 57.4, 104.5, 108.0, 116.1, 119.8, 123.6, 129.2, 129.6, 130.4, 130.5, 131.4, 134.2, 150.0, 151.7, 154.6, 162.8; HRMS calcd for $C_{21}H_{22}N_4O_3H$: 379.1771; found; 379.1772.

4.1.12. 11-[(2-Dimethylamino)ethyl]-2,3-dimethoxy-9-nitro-11*H*-isoquino[4,3-c]cinnolin-12-one (5). Prepared from **16** (220 mg, 0.4 mmol); (25% yield); reaction time 2 h; mp 262–264 °C (dec.); ^{1}H NMR ($CDCl_3$) δ 2.49 (s, 6H), 3.20 (t, 2H, $J=7.0$), 4.11 (s, 3H), 4.20 (s, 3H), 4.70 (t, 2H, $J=7.0$), 7.89 (s, 1H), 8.59 (dd, 1H, $J=9.2$, $J=1.8$), 8.69 (s, 1H), 8.78 (d, 1H, $J=9.0$), 9.91 (d, 1H, $J=1.8$); ^{13}C NMR ($CDCl_3$) δ 46.1, 48.2, 56.5, 56.8, 57.3, 104.5, 108.1, 115.0, 120.0, 122.0, 123.1, 128.5, 131.5, 133.2, 135.4, 147.7, 149.9, 152.4, 154.9, 162.4; IR ($CHCl_3$) 1347, 1533, 1663; HRMS calcd for $C_{21}H_{22}N_5O_5H$: 424.1621; found: 424.1616.

4.1.13. 4-Chloro-6,7-methylenedioxycinnoline (6). 6,7-Methylenedioxy-4-cinnolone (1.0 g, 5.3 mmol) was added in small portions to a stirred mixture of phosphorus pentachloride (1.4 g, 6.7 mmol) and phosporus oxychloride (4 mL, 6.6 mmol) at room temperature. The reaction flask was heated to 80 °C for 4 h, then cooled to room temperature and poured onto 50 g of crushed ice. Following neutralization of the solution with solid sodium acetate, the precipitate was removed by filtration and recrystallized from ethanol to give 800 mg of the chlorocinnoline as an off-white solid, in 73% yield; mp 203.5–204.5 °C; ^{1}H NMR ($CDCl_3$) δ 6.25 (s, 2H), 7.39 (s, 1H), 7.73 (s, 1H), 9.14 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 97.8, 102.9, 105.1, 124.2, 133.4, 144.0, 150.0, 152.3, 152.7; HRMS calcd for $C_9H_5O_2N_2Cl$: 208.0040; found; 208.0042.

4.1.14. 6,7-Methylenedioxy-4-cinnolone. A mixture of 6′-amino-3′,4′-methylenedioxyacetophenone (2.40 g, 13.4 mmol) in concentrated hydrochloric acid (92 mL) and water (13 mL) was cooled to −5 °C and a diazotized by the dropwise addition of a solution of sodium nitrite (0.925 g, 13.4 mmol) in water (4 mL). After stirring for an additional 1 h at −5 °C the mixture was transferred to a bath preheated at 75 °C and left to stir at this temperature overnight. The reaction mixture was cooled to 5 °C to induce crystallization. This material was filtered and then added to 10% aqueous NaOH (100 mL), which was again filtered and dried under vacuum to yield 2.37 g of the cinnoline as a colorless solid, in 93% yield; mp 318–320 °C; ^{1}H NMR (DMSO-d_6) δ 6.21 (s, 2H), 6.97 (s, 1H), 7.30 (s, 1H), 7.63 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 94.9, 100.3, 103.3, 120.1, 139.7, 139.9, 147.4, 153.5, 169.4; HRMS calcd for $C_9H_6O_3N_2$: 190.0378; found: 190.0372.

4.2. General procedure for the formation of 4-amino-6,7-methylenedioxycinnolines (7a–h)

The appropriate primary amine (2 mL/mmol of **6** unless noted otherwise) is added with stirring to intermediate **6**. Copper powder was added in certain reactions as indicated. The reaction was then allowed to stir at 100 °C, unless otherwise noted, for several hours. The reaction mixture was then concentrated in vacuo. The residue was partitioned between $CHCl_3$ and 10%

NaOH. The aqueous layer was repeatedly extracted with $CHCl_3$. All of the $CHCl_3$ solutions (initial partition and extracts) were combined and dried ($MgSO_4$).

4.2.1. N′-(6,7-Methylenedioxycinnolin-4-yl)-*N*,*N*-dimethylethane-1,2-diamine (7a). Prepared from **6** (350 mg, 1.7 mmol); (74% yield); reaction time 3 h; mp 228–230 °C; ^{1}H NMR ($CDCl_3$) δ 2.33 (s, 6H), 2.70 (t, 2H, J = 5.9), 3.38 (m, 2H), 6.15 (s, 2H), 7.03 (s, 1H), 7.56 (s, 1H), 8.53 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 39.5, 45.1, 57.0, 94.7, 102.1, 105.3, 112.7, 128.8, 139.8, 147.8, 149.5, 150.7; HRMS calcd for $C_{13}H_{16}O_2N_4$: 260.1273; found: 260.1267.

4.2.2. N′-(6,7-Methylenedioxycinnolin-4-yl)-*N*,*N*-diethylethane-1,2-diamine (7b). Prepared from **6** (1.0 g, 4.8 mmol); (70% yield); reaction time 3 h; mp 230–232 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (t, 6H, J = 7.2), 2.63 (q, 4H, J = 7.2), 2.84 (t, 2H, J = 5.7), 3.35 (q, 2H, J = 5.7), 5.78 (br, 1H), 6.15 (s, 2H), 6.96 (s, 1H), 7.57 (s, 1H), 8.52 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 12.2, 39.5, 46.6, 50.8, 94.4, 102.0, 105.4, 112.8, 129.0, 139.8, 147.8, 149.5, 150.7; HRMS calcd for $C_{15}H_{20}O_2N_4$: 288.1586; found: 288.1575.

4.2.3. N^2-(6,7-Methylenedioxycinnolin-4-yl)-N^1,N^1-dimethylpropane-1,2-diamine (7c). Prepared from **6** (0.52 g, 2.5 mmol); (42% yield), reaction time 4 h, mp 196–197 °C; ^{1}H NMR (CD_3OD) δ 1.31 (d, 3H, J = 6.6), 2.33 (s, 6H), 2.45 (dd, 1H, J = 5.4, 12.8), 2.74 (dd, 1H, J = 8.2, 12.6), 4.12 (dd, 1H, J = 5.8, 13.8), 6.19 (s, 2H), 7.32 (s, 1H), 7.56 (s, 1H), 8.51 (s, 1H); ^{13}C NMR (CD_3OD) δ 17.1, 44.0, 45.3, 63.5, 95.1, 101.6, 102.0, 112.6, 126.7, 140.8, 149.3, 151.2; HRMS calcd for $C_{14}H_{18}O_2N_4$: 274.1430; found: 274.1429.

4.2.4. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*n*-butylamine (7d). Prepared from **6** (1.0 g, 4.8 mmol), *n*-butylamine (25 mL) and copper powder (250 mg); (32.5% yield); reaction time 18 h at 80 °C, mp 247–248 °C; ^{1}H NMR ($CDCl_3$) δ 1.02 (t, 3H, J = 7.4), 1.50 (m, 2H), 1.73 (m, 2H), 3.40 (m, 2H), 4.59 (s, 1H), 6.14 (s, 2H), 6.96 (s, 1H), 7.57 (s, 1H), 8.59 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.8, 20.2, 29.7, 31.3, 42.8, 94.2, 102.1, 105.3, 112.7, 126.7, 140.6, 149.6, 150.7; HRMS calcd for $C_{13}H_{15}N_3O_2H$: 246.1243; found 246.1237.

4.2.5. 2-[[*N*-(6,7-Methylenedioxycinnolin-4-yl)]amino]methyl]tetrahydrofuran (7e). This was prepared from **6** (500 mg, 2.4 mmol); (78% yield); reaction time 2 h; mp 196–198 °C; ^{1}H NMR ($CDCl_3$) δ 1.74 (m, 1H), 2.11 (m, 3H), 3.30 (m, 1H), 3.58 (m, 1H), 3.92 (m, 2H), 4.29 (m, 1H), 5.22 (br, 1H), 6.12 (s, 2H), 6.98 (s, 1H), 7.52 (s, 1H), 8.54 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 25.9, 29.2, 46.9, 68.4, 76.9, 94.4, 102.2, 105.2, 112.8, 128.7, 139.8, 147.9, 149.6, 150.8; HRMS calcd for $C_{14}H_{15}O_3N_3$: 273.1130; found: 273.1130.

4.2.6. 1-[2-[*N*-(6,7-Methylenedioxycinnolin-4-yl)]amino]-ethylpyrrolidine (7f). This was prepared from **6** (750 mg, 3.5 mmol), 1-(2-aminoethyl)pyrrolidine (3 mL) and copper powder (300 mg); (75% yield); reaction time 18 h at 90 °C; mp 215 °C (dec); ^{1}H NMR ($CDCl_3$) δ 1.85 (m, 4H), 2.63 (m, 4H), 2.90 (t, 2H, J = 6.0), 3.42 (t, 2H, J = 6.0), 5.63 (s, 1H), 6.14 (s, 2H), 7.04 (s, 1H), 7.57 (s, 1H), 8.53 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 23.9, 42.0, 54.5, 54.7, 97.0, 102.9, 104.4, 112.7, 126.8, 140.8, 149.3, 151.0; HRMS calcd for $C_{15}H_{18}N_4O_2$: 293.1590; found 293.1579.

4.2.7. 1-[2-[*N*-(6,7-Methylenedioxycinnolin-4-yl)]amino]-ethylpiperidine (7g). This was prepared from **6** (1.04 g, 5.0 mmol); (37% yield); reaction time 2 h; mp 238–239 °C; ^{1}H NMR (CD_3OD) δ 1.56 (d, 2H, J = 5.2), 1.70 (d, 2H, J = 4.6), 2.87 (t, 2H, J = 7.0), 3.65 (t, 2H, J = 6.6), 6.20 (s, 2H), 7.32 (s, 1H), 7.43 (s, 1H), 8.46 (s, 1H); ^{13}C NMR (CD_3OD) δ 23.1, 24.7, 38.5, 53.6, 56.1, 94.7, 101.7, 102.1, 112.4, 126.6, 141.1, 146.7, 149.4, 151.2 ($CDCl_3$); HRMS calcd for $C_{16}H_{20}N_4O_2H$: 300.1586; found 300.1586.

4.2.8. 1-[2-[*N*-(6,7-Methylenedioxycinnolin-4-yl)]amino]-ethyl-4-methylpiperazine (7h). This was prepared from **6** (1.0 g, 4.8 mmol); (52% yield); reaction time 2 h at 120 °C; mp 227–229 °C; ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H), 2.48 (m, 4H), 2.54 (m, 4H), 2.76 (t, 2H, J = 7.2), 3.37 (m, 2H), 5.73 (br, 1H), 6.11 (s, 2H), 6.99 (s, 1H), 7.51 (s, 1H), 8.47 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 38.8, 46.1, 52.7, 55.3, 55.6, 94.5, 102.1, 105.2, 112.7, 128.7, 139.4, 147.8, 149.5, 150.7; HRMS calcd for $C_{16}H_{21}N_5O_2$: 315.1695; found: 315.1699.

4.3. General procedure for the formation of 2-Iodo-4,5-dimethoxybenzamides (8a–h)

A 2.0 M solution of oxalyl chloride in CH_2Cl_2 (1.3 equiv) was added to a solution of 2-iodo-4,5-dimethoxybenzoic acid (1.0 equiv) in anhydrous CH_2Cl_2 (60 mL per 10 mmol benzoic acid) and the solution stirred at reflux for 3 h. The mixture was allowed to cool and was then concentrated to dryness in vacuo. To the residue was added a solution of the appropriate 4-amino-6,7-methylenedioxycinnoline (1.0 equiv), triethylamine (2 equiv) in CH_2Cl_2 (60 mL per 4 mmol aminocinnoline). The reaction mixture was then stirred at reflux under N_2. The reaction mixture was cooled and washed with satd $NaHCO_3$. The reaction mixture was concentrated in vacuo. The product was isolated using column chromatography for those analogues not possessing a tertiary amine. In the case of those analogues containing a tertiary amine, the residue was extracted with 3% HCl. The aqueous layer was neutralized with 20% NaOH and extracted with $CHCl_3$, dried ($MgSO_4$) and evaporated.

4.3.1. *N*-(6,7-Methylenedioxycinnnolin-4-yl)-*N*-[(2-*N*,*N*-dimethylamino)ethyl] - 2 - iodo - 4,5 - dimethoxybenzamide (8a). This was prepared from **7a** (1.0 g, 3.84 mmol); (75% yield); reaction time 4 h; IR ($CHCl_3$) 1655; ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 6H), 2.53 (m, 2H), 3.43 (s, 3H), 3.75 (s, 3H), 3.97 (m, 1H), 4.44 (m, 1H), 6.24 (s, 1H), 6.25 (s, 1H), 6.43 (s, 1H), 7.02 (s, 1H), 7.43 (s, 1H), 7.68 (s, 1H), 9.18 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.5, 47.1, 55.7, 56.1, 56.7, 82.8, 96.7, 102.9, 105.4, 110.6, 121.9, 123.2, 133.1, 136.0, 144.8, 148.2, 149.9, 150.9, 151.7, 152.4, 169.8; HRMS calcd for $C_{22}H_{23}O_5N_4IH$: 551.0791; found: 551.0795.

4.3.2. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[2-(*N*,*N*-diethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (8b). This was prepared from **7b** (640 mg, 2.2 mmol); (87% yield); reaction time 16 h; IR ($CHCl_3$) 1656; ^1H NMR ($CDCl_3$) δ 0.92 (t, 6H, $J=7.0$), 2.50 (q, 4H, $J=7.0$), 2.80 (t, 2H, $J=6.8$), 3.39 (s, 3H), 3.71 (s, 3H), 3.94 (m, 1H), 4.41 (m, 1H), 6.21 (d, 2H, $J=1.4$), 6.39 (s, 1H), 7.01 (s, 1H), 7.39 (s, 1H), 7.64 (s, 1H), 9.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 11.6, 46.9, 47.8, 51.1, 55.7, 56.1, 82.9, 96.9, 102.9, 105.5, 110.9, 122.1, 122.9, 133.0, 136.5, 144.9, 148.3, 150.1, 150.9, 151.7, 152.3, 169.8; HRMS calcd for $C_{24}H_{27}O_5N_4IH$: 579.1105; found: 579.1105.

4.3.3. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)-1-methylethyl)-2-iodo-4,5-dimethoxybenzamide (8c). This was prepared from **7c** (240 mg, 0.87 mmol); (83% yield); reaction time 16 h; mp 110–111 °C; ^1H NMR ($CDCl_3$) was a mixture of atropisomer isomers: #1 δ 1.03–1.36 (m, 3H), 2.21–2.37 (m, 6H), 2.74–3.07 (m, 1H), 3.43–3.65 (m, 6H), 3.84–3.91 (m, 1H), 5.15 (m, 1H), 6.18 (s, 2H), 6.59 (s, 1H), 6.91 (s, 1H), 7.56 (s, 1H), 8.04 (s, 1H), 9.34 (s, 1H); isomer #2 δ 1.03–1.36 (m, 3H), 2.31-2.37 (m, 6H), 2.74–3.07 (m, 1H), 3.43–3.65 (m, 6H), 3.84–3.91 (m,1H), 5.15 (m, 1H), 6.18 (s, 2H), 6.59 (s, 1H), 6.91 (s, 1H), 7.56 (s, 1H), 8.04 (s, 1H), 9.34 (s, 1H); HRMS calcd for $C_{23}H_{25}O_5N_4IH$: 565.0870; found: 565.0926.

4.3.4. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-(*n*-butyl)-2-iodo-4,5-dimethoxybenzamide (8d). This was prepared from **7d** (350 mg, 1.4 mmol); (19% yield) reaction time 18 h; mp 133–134 °C; IR (KBr) 1654; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, $J=7.2$), 1.20–1.90 (m, 4H), 3.33 (s, 3H), 3.68 (s, 3H), 3.90 (m, 1H), 4.35 (m, 1H), 6.19 (d, 2H, $J=3.2$), 6.34 (s, 1H), 6.98 (s, 1H), 7.25 (s, 1H), 7.62 (s, 1H), 9.01 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.7, 20.1, 30.0, 49.4, 55.7, 56.1, 82.9, 96.5, 102.9, 105.6, 110.5, 121.9, 133.1, 148.3, 150.0, 151.8, 152.5, 169.7; HRMS calcd for $C_{22}H_{22}IN_3O_5Li$ 542.0764; found 542.0757.

4.3.5. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[2-(tetrahydrofuranyl)methyl]-2-iodo-4,5-dimethoxybenzamide (8e). This was prepared from **7e** (400 mg, 1.5 mmol); (34% yield); reaction time 16 h; IR ($CHCl_3$) 1654; ^1H NMR, a mixture of atropisomers, ($CDCl_3$) isomer #1: δ 1.94 (m, 4H), 3.70 (m, 4H), 3.73 (s, 3H), 3.94 (s, 3H), 4.34 (m, 1H) 6.23 (s, 2H), 7.00 (s, 1H), 7.40 (s, 1H), 7.70 (s, 1H), 9.31 (s, 1H); isomer #2 δ 1.94 (m, 4H), 3.70 (m, 4H), 3.73 (s, 3H), 3.94 (s, 3H), 4.34 (m, 1H) 6.46 (s, 2H), 7.36 (s, H), 7.49 (s, 1H), 7.65 (s, 1H), 9.17 (s, 1H); HRMS calcd for $C_{23}H_{22}O_6N_3IH$: 564.0632; found: 564.0650.

4.3.6. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[(2-pyrrolidin-1-yl)ethyl]-2-iodo-4,5-dimethoxybenzamide (8f). This was prepared from **7f** (400 mg, 0.4 mmol); (42% yield); reaction time 4 h; IR (KBr) 1655; ^1H NMR ($CDCl_3$) δ 1.60 (m, 4H), 2.40 (m, 4H), 2.67 (m, 2H), 3.28 (s, 3H), 3.60 (s, 3H), 4.32 (m, 1H), 6.11 (d, 2H, $J=2.2$), 6.32 (s, 1H), 6.91 (s, 1H), 7.37 (s, 1H), 7.50 (s 1H), 9.04 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 23.6, 29.7, 47.6, 52.9, 53.9, 55.7, 56.0, 56.4, 82.8, 96.7, 102.9, 105.4, 110.6, 121.9, 123.1, 132.8, 135.9, 144.7, 148.2, 149.9, 150.9, 151.7, 152.4, 169.9; HRMS calcd for $C_{24}H_{25}IN_4O_5HLi$ 584.1108; found 584.1115.

4.3.7. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[2-(piperidin-1-yl)ethyl]-2-iodo-4,5-dimethoxybenzamide (8g). This was prepared from **7g** (500 mg, 1.66 mmol); (85% yield); reaction time 16 h; mp 93–94 °C; IR (KBr) 1655; ^1H NMR ($CDCl_3$) δ 1.43 (m, 6H), 2.35 (m, 4H), 2.50–2.71 (m, 2H), 3.43 (s, 3H), 3.73 (s, 3H), 3.78–3.93 (m, 1H), 4.32–4.42 (m, 1H), 6.22 (d, 2H, $J=1.6$), 6.42 (s, 1H), 7.02 (s, 1H), 7.47 (s, 1H), 7.66 (s, 1H), 9.19 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.3, 25.9, 46.0, 46.4, 54.5, 55.6, 56.0, 56.4, 82.9, 97.0, 102.8, 105.3, 110.8, 122.0, 113.7, 123.2, 133.1, 136.3, 145.0, 148.2, 149.9, 150.8, 151.6, 152.1, 169.8; HRMS calcd for $C_{23}H_{25}IN_4O_5H$: 591.1105; found 591.1108.

4.3.8. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[2-(4-methyl-1-piperazinyl)ethyl]-2-iodo-4,5-dimethoxybenzamide (8h). This was prepared from **7h** (700 mg, 2.2 mmol) (82% yield); ^1H NMR ($CDCl_3$) δ 2.20 (s, 3H), 2.32 (m, 4H), 2.42 (m, 4H), 3.37 (s, 3H), 3.68 (s, 3H), 3.86 (m, 3H), 4.34 (m, 1H), 6.18 (s, 1H), 6.19 (s, 1H), 6.37 (s, 1H), 6.97 (s, 1H), 7.42 (s, 1H), 7.60 (s, 1H), 9.13 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.9, 46.1, 53.0, 55.1, 55.6, 55.7, 56.1, 82.9, 97.0, 102.9, 105.3, 110.7, 121.9, 123.2, 132.9, 136.1, 145.0, 148.2, 149.9, 150.9, 151.7, 152.3, 169.8; IR ($CHCl_3$) 1654; HRMS calcd for $C_{25}H_{28}IN_5O_5H$: 606.1213; found 606.1213.

4.3.9. 4-Chlorocinnoline (9). A mixture of 4-hydroxycinnoline (2.0 g, 13.7 mmol), phosphorus oxychloride (1.94 mL, 20.5 mmol), and pyridine (0.33 mL, 4.1 mmol) in chlorobenzene (50 mL) was refluxed for 1 h. Then the mixture was cooled and the solvent evaporated under vacuum, water was added, and the mixture was neutralized with solid sodium bicarbonate and extracted with chloroform (3×100 mL), washed with water (3×100 mL), dried ($MgSO_4$) and evaporated under vacuum, giving 1.84 g, in 82% yield; mp 76–77 °C (lit.[28] mp 78 °C); ^1H NMR ($CDCl_3$) δ 7.91 (m, 2H), 8.20 (dd, 1H, $J=6.2$, $J=2.1$), 8.57 (dd, 1H, $J=6.6$, $J=1.8$), 9.34 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 123.0, 124.9, 130.2, 131.6, 132.3, 133.8, 134.9, 144.4.

4.3.10. 4-[2-(Dimethylamino)ethylamino]cinnoline (11). Intermediate **9** (1.0 g, 6.1 mmol) was stirred in neat refluxing *N*,*N*-dimethylethylenediamine (6.25 g, 70.9 mmol) for 3 h, then the mixture was cooled and the solvent evaporated under reduced pressure. The crude material was partitioned between water (100 mL) and chloroform (100 mL), and the aqueous phase was washed with chloroform (2×100 mL). The combined organic phases were washed with brine (75 mL), dried ($MgSO_4$) and evaporated under vacuum, giving 890 mg, in 68% yield; mp 146–148 °C; ^1H NMR ($CDCl_3$) δ 2.31 (s, 6H), 2.69 (t, 2H, $J=5.9$), 3.88 (m, 2H), 6.08 (br, 1H), 7.57 (ddd, 1H, $J=8.3$, $J=7.4$, $J=0.8$), 7.72 (ddd, 1H, $J=8.4$, $J=7.4$, $J=0.6$), 7.82 (dd, 1H, $J=8.3$, $J=0.6$), 8.30 (dd, $J=8.4$, $J=0.8$), 8.63 (s, 1H); ^{13}C NMR ($CDCl_3$) 39.6, 45.1, 57.0, 115.8, 119.2, 128.2, 128.7, 129.5, 129.9, 139.9, 148.7; HRMS calcd for $C_{12}H_{16}N_4$: 216.1375; found 216.1376.

4.3.11. *N*′-(6-Nitrocinnolin-4-yl)-*N*,*N*-dimethylethane-1,2-diamine (12). A mixture of **10** (2.0 g, 7.5 mmol) and

N,*N*-dimethylethylenediamine (1.33 g, 15.2 mmol) in DMF (20 mL) was heated to 90 °C with stirring for 1 h. Then the mixture was cooled and the solvent was removed under vacuum. The residue was partitioned between 10% NaOH (150 mL) and $CHCl_3$ (100 mL), and the aqueous phase was extracted with $CHCl_3$ (4×100 mL). The combined organic extracts were washed with 10% NaOH (2×150 mL), dried ($MgSO_4$), and evaporated under vacuum, yielding 1.4 g, in 72% yield; mp 200–202 °C; IR ($CHCl_3$) 1345, 1518, 3349; ^{1}H NMR ($CDCl_3$) δ 2.36 (s, 3H), 2.77 (t, 2H, J = 5.9), 3.54 (m, 2H), 6.87 (br, 1H), 8.40 (m, 2H), 8.82 (s, 1H), 8.95 (d, 1H, J = 1.8); ^{13}C NMR ($CDCl_3$) δ 40.0, 45.2, 56.7, 114.5, 118.2, 123.5, 130.4, 131.4, 141.5, 145.7, 149.3; HRMS calcd for $C_{12}H_{15}N_5O_2$: 261.1226; found: 261.1233.

4.3.12. *N*-(6,7-Methylenedioxycinnolin-4-yl)-*N*-[(2-*N*,*N*-dimethylamino)ethyl]-2-iodobenzamide (13). Oxalyl chloride (1.05 g, 8.1 mmol) was added to a solution of *o*-iodobenzoic acid (0.57 g, 2.3 mmol) in methylene chloride (20 mL) under nitrogen, and the stirred mixture was refluxed for 4 h. Then the mixture was concentrated to dryness under vacuum. The acid chloride was redissolved in methylene chloride (20 mL) and added to a solution of **7a** and triethylamine (2.0 g, 20.0 mmol) in methylene chloride (20 mL). The reaction was stirred at reflux under nitrogen for 2 h. The mixture was then cooled and washed with a saturated solution of sodium bicarbonate (3×75 mL), and extracted into dilute HCl (3×75 mL), and the combined aqueous extracts were washed with chloroform (75 mL) and basified using 30% NaOH, then extracted with chloroform (3×75 mL). The combined organic extracts were washed with brine (100 mL), dried (anhydrous $MgSO_4$), and concentrated in vacuo, to give 652 mg as a gum, in 70% yield; IR ($CHCl_3$) 1658; ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 6H), 2.58 (m, 2H), 3.59 (m, 1H), 4.53 (m, 1H), 6.21 (s, 1H), 6.23 (s, 1H), 6.81 (m, 3H), 7.36 (s, 1H), 7.61 (s, 1H), 7.68 (m, 1H), 9.11 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.4, 46.8, 56.7, 93.7, 96.7, 102.9, 105.4, 123.1, 126.8, 127.4, 128.4, 135.7, 139.9, 140.9, 144.9, 150.1, 151.9, 152.6, 169.9; HRMS calcd for $C_{20}H_{19}O_3N_4IH$: 491.0581; found 491.0592.

4.3.13. *N*-(Cinnolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)-ethyl]-2-iodobenzamide (14). This was prepared from **11** (740 mg, 3.4 mmol); (33% yield); reaction time 18 h at 50 °C from the acid chloride prepared using 20 mmol of oxalyl chloride and 4.0 mmol of 2-iodobenzoic acid; IR ($CHCl_3$) 1660; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6H), 2.43–2.53 (m, 1H), 2.56–2.66 (m, 1H), 3.52–3.68 (m, 1H), 4.51–4.64 (m, 1H) 6.69–6.76 (m, 3H), 7.30–7.57 (m, 2H), 7.75–7.82 (m, 3H), 8.09–8.14 (m, 1H), 8.39–8.44 (m, 1H), 9.29 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.4, 47.1, 56.7, 93.8, 122.1, 123.4, 126.9, 127.3, 128.4, 130.4, 130.6, 131.2, 136.2, 139.7, 140.8, 145.1, 151.6, 169.7; HRMS calcd for $C_{19}H_{19}IN_4OH$: 447.0682; found 447.0687.

4.3.14. *N*-(Cinnolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (15). Oxalyl chloride (2.1 g, 16.2 mmol) was added to a solution of 3,4-dimethoxy-6-iodobenzoic acid (1.5 g, 4.87 mmol) in anhydrous methylene chloride (40 mL), and the stirred mixture was refluxed for 4 h. The mixture was then concentrated to dryness under reduced pressure. The acid chloride was redissolved in 40 mL of anhydrous methylene chloride, and this solution was added to a solution of **11** (870 mg, 4.05 mmol) and triethylamine (4.0 g, 40.0 mmol) in methylene chloride (30 mL), and the resulting mixture was stirred at reflux overnight. The reaction mix was cooled and washed with saturated sodium bicarbonate (3×75 mL), and brine (75 mL), dried ($MgSO_4$) and evaporated, and the crude product mixture was chromatographed in 98:2 chloroform–methanol, to provide 165 mg of the desired amide, in 8% yield; IR ($CHCl_3$) 1658; ^{1}H NMR ($CDCl_3$) δ 2.21 (s, 6H), 2.63 (m, 2H), 3.19 (s, 3H), 3.70 (s, 3H), 3.94 (m, 1H), 4.59 (m, 1H), 6.28 (s, 1H), 7.01 (s, 1H), 7.88 (m, 2H), 8.20 (dd, 1H, J = 6.6, J = 3.4), 8.54 (dd, 1H, J = 6.6, J = 3.0), 9.35 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 45.5, 47.3, 55.4, 56.1, 56.8, 82.9, 110.4, 121.9, 122.0, 123.8, 130.7, 131.0, 132.0, 133.0, 136.6, 145.2, 148.2, 149.9, 151.7, 169.9; HRMS calcd for $C_{21}H_{23}IN_4O_3H$: 507.0894; found 507.0880.

4.3.15. *N*-(6-Nitrocinnolin-4-yl)-*N*-[2-(*N*,*N*-dimethylamino)ethyl]-2-iodo-4,5-dimethoxybenzamide (16). Oxalyl chloride (3.0 g, 23.2 mmol) was added to a mixture of 2-iodo-4,5-dimethoxybenzoic acid (1.5 g, 4.9 mmol) in methylene chloride (40 mL), and the stirred mixture was heated to reflux under nitrogen for 4 h. The mixture was concentrated under reduced pressure and the acid chloride was redissolved in methylene chloride (40 mL) and added to a mixture of **12** (1.0 g, 4.0 mmol) and TEA (2.5 g, 25.0 mmol) in methylene chloride (40 mL). The resulting mixture was heated to reflux overnight and then cooled and washed with saturated sodium bicarbonate (3×100 mL) and brine (150 mL), dried ($MgSO_4$) and evaporated, and the residue was chromatographed in 99:1 $CHCl_3$–MeOH to provide 590 mg as a glue, in 29% yield; IR ($CHCl_3$) 1654; ^{1}H NMR ($CDCl_3$) δ 2.11 (s, 6H), 2.58 (m, 2H), 3.40 (s, 3H), 3.64 (s, 3H), 3.85 (m, 1H), 4.16 (m, 1H), 6.51 (s, 1H), 6.85 (s, 1H), 8.50 (m, 1H), 8.60 (m, 1H), 9.28 (s, 1H), 9.61 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 45.2, 48.1, 52.3, 56.2, 57.0, 82.5, 111.3, 121.0, 121.7, 123.8, 124.3, 131.0, 132.7, 138.2, 145.8, 148.8, 149.0, 150.2, 151.4, 169.6; HRMS calcd for $C_{21}H_{23}IN_5O_5H$: 552.0744; found: 552.0743.

4.4. Topoisomerase-mediated DNA cleavage assays

Human topoisomerase I was expressed in *Escherichia coli* and isolated as a recombinant fusion protein using a T7 expression system as described previously.[14] Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described.[34] The 3′ end-labeling of the plasmid was accomplished by digestion with a restriction enzyme followed by end-filling with Klenow polymerase as previously described.[35] The cleavage assays were performed as previously reported.[36,37] The drug and the DNA in presence of topoisomerase I was incubated for 30 min at room temperature. The reactions were terminated by the addition of 5 μL of 5% SDS and 1 mg/mL protein kinase K with an additional

1 h of incubation at 37 °C. Samples were then alkali denatured by the addition of NaOH, EDTA, sucrose, and bromophenol blue to final concentrations of 75 mM, 2.5%, and 0.05 mg/mL, respectively, prior to loading onto a neutral agarose gel. After development of the gels, typically 24-h exposure was used to obtain autoradiograms outlining the extent of DNA fragmentation. Topoisomerase I-mediated DNA cleavage values are reported as Relative Effective Concentration (REC), that is concentrations relative to topotecan, whose value is arbitrarily assumed as 1.0, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I.

4.5. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA).[38–40] The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 were provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan).[40] The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in RPMI medium supplemented with 10% heat- inactivated fetal bovine serum, L-glutamine (2 mM), penicillin (100 U/mL), and streptomycin (0.1 mg/mL). Each well was plated with approximately 2000 RPMI8402 cells; 4000 CPT-K5 cells; 1000 P388 cells and 2500 P388/CPT45 cells. For determination of IC_{50}, cells were exposed continuously for 4 days to varying concentrations of drug, and MTT assays were performed at the end of the fourth day. Each assay was performed with a control that did not contain any drug. All assays were performed at least twice in six replicate wells.

4.6. Human tumor xenograft

Two bioassays were performed using female NCR/NU NU mice of approximately 9 weeks of age as obtained from Taconic Farms, Inc. (Germantown, NY, USA). Mice were housed four per cage in laminar flow HEPA filtered microisolator caging (Allentown Caging Equipment Co., Allentown, NJ, USA). Mice were fed Purina autoclavable breeder chow #5021 and given drinking water, purified by reverse-osmosis, ad libitum. Five days after delivery, the mice were inoculated on the right flank with 1.5×10^6 MDA-MB-435 tumor cells in 0.1 mL of RPMI 1640 Media by sc injection (25 gauge needle$\times$5/8″). The MDA-MB-435 cells were grown in 75 cm^2 flasks using RPMI 1640 Media and 10% fetal bovine serum. Tumors were of sufficient size at 19–20 days after inoculation. Tumor-bearing mice were matched in each experimental group based on tumor volume, that is the mice with the larger tumor volumes were placed within each experimental group. Tumor volume was calculated by measuring the tumor with a microcaliper. The length (l) is the maximum two-dimensional distance of the tumor and the width (w) is the maximum distance perpendicular to this length measured in mm. Tumor volume was calculated using the formula $(l\times w^2)/2$. Every mouse in this study was weighed individually on a daily basis. Tumor volume was determined for each individual mouse every other day.

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