Bioorganic & Medicinal Chemistry 16 (2008) 7824–7831

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Syntheses and biological evaluation of topoisomerase I-targeting agents related to 11-[2-(*N*,*N*-dimethylamino)ethyl]-2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-one (ARC-31)

Mavurapu Satyanarayana [a], Wei Feng [a], Liang Cheng [a], Angela A. Liu [b], Yuan-Chin Tsai [b], Leroy F. Liu [b,c], Edmond J. LaVoie [a,c,*]

[a] Department of Pharmaceutical Chemistry, Rutgers, Ernest Mario School of Pharmacy, The State University of New Jersey, Piscataway, NJ 08854-8020, USA
[b] Department of Pharmacology, The University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, Piscataway, NJ 08854-8020, USA
[c] The Cancer Institute of New Jersey, New Brunswick, NJ 08901, USA

ARTICLE INFO

Article history:
Received 20 May 2008
Revised 23 June 2008
Accepted 24 June 2008
Available online 26 June 2008

Keywords:
Topoisomerase
Cinnolines
Isoquino[4,3-*c*]cinnolin-12-ones
Cytotoxicity
Antitumor
Multidrug resistance
Athymic mice
Human tumor xenografts

ABSTRACT

Several 11-ethyl-2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-ones with varied functionality on the ethyl substituent have exhibited potent topoisomerase I (TOP1) targeting activity and antitumor activity. The influence of various polar substituents at the 2-position of the 11-ethyl substituent, including *N*-methylamine, *N*-isopropylamine, hydroxyl, and hydroxylamino groups, on TOP1-targeting activity and cytotoxicity was assessed. The *N*-methylamine and *N*-isopropylamine derivatives were also evaluated as antitumor agents in athymic nude mice with MDA-MB-435 human tumor xenografts. Both compounds were active as antitumor agents upon either parenteral or oral administration.

1. Introduction

Topoisomerases are ubiquitous enzymes that participate in processes such as DNA replication, repair, transcription, and recombination as well as chromosome condensation and genomic stability.[1–3] Topoisomerase I (TOP1) has been the target of several antitumor agents based upon their ability to stabilize the enzyme–DNA cleavage complex, which results in DNA damage and ultimately cell death.[4,5]

Camptothecin (CPT) and its derivatives were among the first compounds identified as TOP1-targeting agents. Extensive studies on camptothecin analogs resulted in the development of two clinical TOP1-targeting drugs, topotecan (Hycamtin®) and irinotecan (CPT-11/Camptosar®). These clinical agents have the camptothecin ring system, which has incorporated within its structure a δ-lactone. Hydrolysis of this lactone results in an inactive derivative that possesses high affinity for human serum albumin.[5–7] The metabolic instability of this lactone and the observation that both topotecan and irinotecan are substrates for efflux transporters associated with multidrug resistance[8–11] have prompted studies on the further development of TOP1-targeting agents for use in cancer chemotherapy.

ARC-111 (**1**, Fig. 1) is a non-camptothecin TOP1-targeting agent that has potent antitumor activity when administered orally or parenterally to athymic nude mice with several human tumor xenografts. Extensive studies have been performed to assess the TOP1-targeting activity and cytotoxicity of several dibenzo[*c*,*h*][1,6]naphthyridin-6-ones related to ARC-111.[12–15] Studies from our laboratory have also demonstrated that similarly substituted dibenzo[*c*,*h*]cinnolines and 11*H*-isoquino[4,3-*c*]cinnolin-12-ones are also highly active.[12,16,17] While 11-[2-(*N*,*N*-dimethylamino)ethyl]-2,3-dimethoxy-8,9-methylenedioxy-11*H*-isoquino-[4,3-*c*]cinnolin-12-one (ARC-31, **2**, Fig. 1) possesses at least similar TOP1-targeting activity and cytotoxicity to ARC-111 and CPT, dose limiting toxicity did appear to constrain its in vivo efficacy as an antitumor agent.[17] In this study both the *N*-methylamine and *N*-isopropylamine analogs of **2** (ARC-31) were synthesized and their

* Corresponding author at present address: Department of Pharmaceutical Chemistry, Ernest School of Pharmacy, Rutgers University, 160 Frelinghuysen Road, Piscataway, NJ 08854, USA. Tel.: +1 732 445 2674; fax: +1 732 445 6312.
E-mail address: elavoie@rci.rutgers.edu (E.J. LaVoie).

0968-0896/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2008.06.046

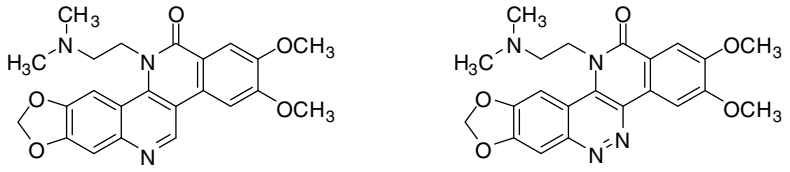


Figure 1. Structures of ARC-111 and ARC-31.

biological activities evaluated. In an effort to further explore whether a less toxic analog could be developed with improved efficacy, a number of analogs related to **2** were synthesized wherein the 11-ethyl substituent was substituted at its 2-position with varied polar substituents. Hydrophilic camptothecin analogs that incorporated monohydroxy-, dihydroxylmethyl, and trihydroxymethylaminoalkyl group exhibited increased ternary complex stability and could inhibit tumor growth in vitro and in vivo.[18] In view of these data, we also prepared the analogs that had in place of the *N*,*N*-dimethyl substituent at the 2-position of the 11-ethyl substituent of **2** a tris(hydroxymethyl)amino (THMA) group, a hydroxy, a 1*H*-imidazol-1-yl, or a[2-(*N*,*N*-dimethylamino)ethyl]methylamino substituent. Methods for the preparation of these analogs via the trimethylammonium derivatives of ARC-31 were recently reported.[19]

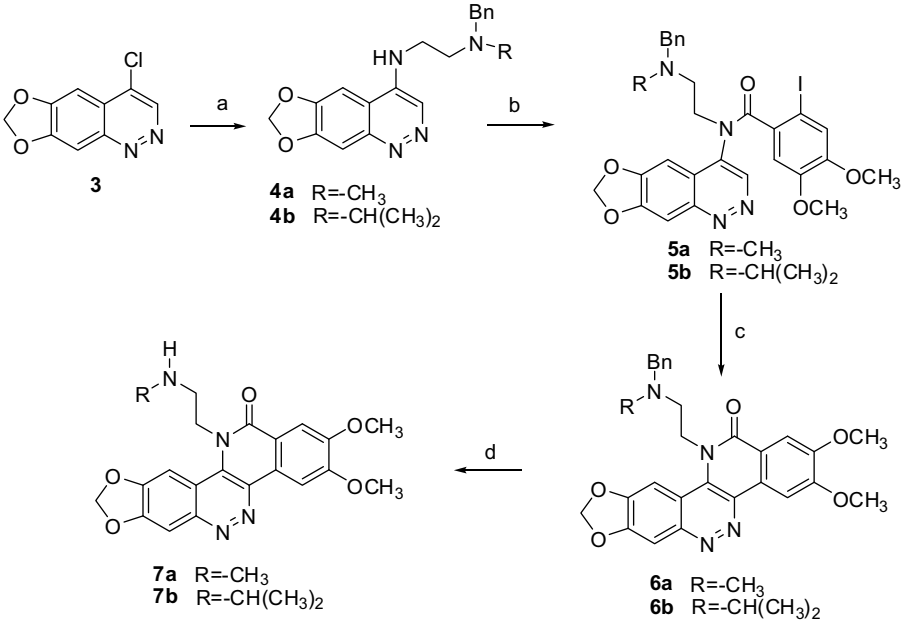
1.1. Chemistry

The synthetic approach for the preparation of 11-[*N*-methylaminoethyl] and 11-[*N*-isopropylaminoethyl] analogs of ARC-31 employed similar methodology to that previously described (Scheme 1).[12,13] Reaction of 4-chloro-6,7-methylenedioxycinnoline 3 with the *N*-benzyl-*N*-methylethylenediamine or *N*-benzyl-*N*-isopropylethylenediamine provided the appropriately substituted 4-[(2-alkylamino)ethylamino]cinnoline **4a** and **4b**. Treatment of these 4-aminocinnoline derivatives with the crude acid chloride prepared from 2-iodo-4,5-dimethoxybenzoic acid gave the tertiary amide intermediates **5a** and **5b**, which under Heck cyclization conditions provided the 11*H*-isoquinolin[4,3-*c*]cinnolin-12-ones **6a** and **6b.** The *N*-benzyl groups on **6a** and **6b** were removed by treatment of a solution of each tertiary amine in acetic acid and formic acid with palladium black at room temperature to give the secondary amine **7a** and **7b**.

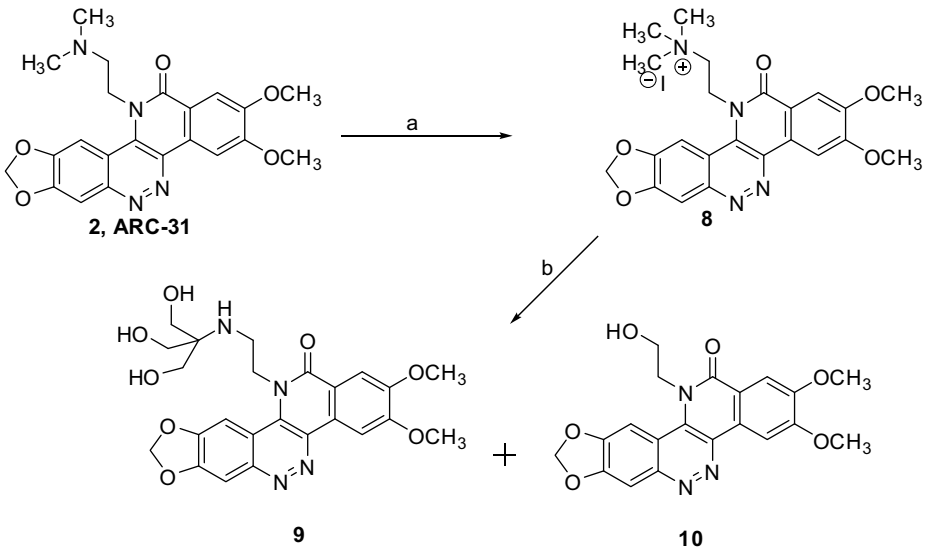
It was previously reported that the trimethylammonium iodide **8** is a useful intermediate for the preparation of several additional analogs of ARC-31.[19] Treatment of **2** with methyl iodide resulted in the formation of **8**, which when heated with tris(hydroxymethyl)aminomethane in DMSO provided **9**, together with the 11-(2-hydroxyethyl) derivative, **10**, as a by-product (Scheme 2). Similarly, 8 reacted with imidazole or *N*,*N*,*N*′-trimethylethylenediamine to provide **11** and **12** (Scheme 3).

2. Results and discussion

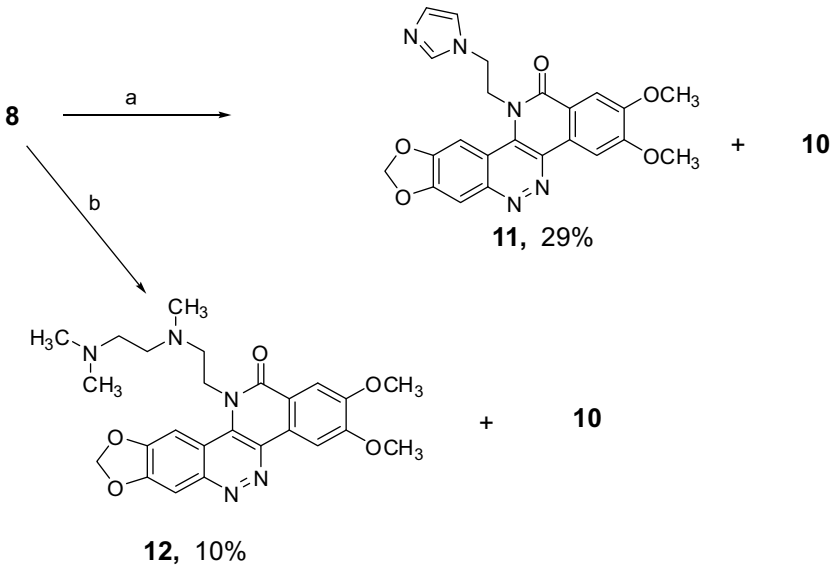
The TOP1-targeting activity of derivatives of 11*H*-isoquino[4,3-*c*]cinnolin-12-one relative to **1**, **2**, and CPT is listed in Table 1. All of the compounds evaluated in this study exhibited potent TOP1-targeting activity. Compounds **7a** and **7b** were significantly more potent than CPT as TOP1-targeting agents causing extensive DNA fragmentation in the sub-cellular cleavage assay. As indicated by the data provided for **6a** and **6b,** the presence of a *N*-benzyl substituent is associated with a decrease in TOP1-targeting activity. Despite the varied substituents on the 11-ethyl substituent in the case of compounds **9–12**, there were no dramatic differences in their intrinsic TOP1-targeting activity relative to CPT or ARC-31.



Scheme 1. Synthesis of 11*H*-isoquinolin[4,3-*c*]cinnolin-12-ones **6a**–**b** and **7a**–**b.** Reagents and conditions: (a) amine, Cu, 100 °C; (b) 4,5-dimethoxy-2-iodobenzoic acid, oxalyl chloride, DCM, TEA, reflux; (c) $Pd(OAc)_2$, P(o-tolyl)$_3$, Ag_2CO_3, DMF, 160 °C; (d) acetic acid, formic acid, Pd, rt.



Scheme 2. Formation of **9** and the by-product **10**. Reagents and conditions: (a) methyl iodide, DCM, Methanol, rt; (b) tris(hydroxymethyl)aminomethane, DMSO, 150 °C, 4 h.



Scheme 3. Synthesis of **11** and **12** from the trimethylammonium intermediate **8**. Reagents and conditions: (a) imidazole, DMSO, 150 °C, 4 h; (b) *N,N,N′*-trimethylethylenediamine, DMSO, 150 °C, 1 h.

Table 1
Relative TOP1-targeting activity and cytotoxicity of ARC-31 analogs

Compound	TOP1-mediated cleavage IC_{10} (nM)[a]	Cytotoxicity IC_{50} (μM)			
		RPMI8402	CPT-K5	P388	P388/CPT45
CPT	0.2	0.005	61	0.009	>10
1, ARC-111	0.3	0.002	0.90	0.001	0.23
2, ARC-31	0.3	0.002	0.74	0.002	0.23
6a	0.4	0.04	>10	0.025	2.8
6b	0.5	0.045	3.3	0.021	2.3
7a	0.03	0.003	0.23	0.001	0.2
7b	0.08	0.003	0.3	0.002	0.11
9	0.2	0.065	5.7	0.04	3.0
10	0.3	0.013	1.3	0.012	0.7
11	0.1	0.4	>10	0.19	>10
12	0.4	0.033	2.2	0.019	0.3

[a] Topoisomerase I cleavage values are reported as REC, relative effective concentration; these are concentrations relative to, topotecan; whose value is arbitrarily assumed as 1, that are able to produce 10% cleavage of the plasmid DNA in the presence of human topoisomerase I.[17]

Table 2
Cytotoxicity in KB3-1 and its multidrug-resistant cell lines, KBV-1 and KBH5.0

Compound	Cytotoxicity IC_{50} (μM)		
	KB3-1 wt	KBV-1 MDR1$^+$	KBH5.0 BCRP$^+$
CPT	0.015	0.025	0.026
1, ARC-111	0.005	0.015	0.012
2, ARC-31	0.005	0.004	0.004
6a	0.035	0.085	0.04
6b	0.016	0.075	0.04
7a	0.0007	0.023	0.018
7b	0.0005	0.007	0.002
9	0.05	0.28	0.4
10	0.01	0.018	0.055
11	0.38	3.3	3.3
12	0.017	0.18	0.25

The relative cytotoxic activities of these 11*H*-isoquino[4,3-*c*]cinnolin-12-ones in RPMI8402 and P388, as well as their respective camptothecin resistant variants, CPT-K5 and P388/CPT-45 are provided in Table 1. Both **7a** and **7b** exhibited comparable cytotoxicity to **1** and **2**. The *N*-benzyl derivatives **6a** and **6b** were at least an order of magnitude less cytotoxic toward these cell lines, which does correlate with their relative activity as TOP1-targeting agents as compared to other 11*H*-isoquino[4,3-*c*]cinnolin-12-ones. In general, the cytotoxic activities of **9**, **10**, and **12** were consistent with their potent TOP1-targeting activity. However, the 1*H*-imidazol-1-yl derivative **11** was substantially less cytotoxic than what would be expected based upon its TOP1-targeting activity. Several factors such as DNA and protein binding could affect intrinsic TOP1-targeting activity. In addition, DNA binding and physicochemical differences that affect cellular absorption can influence the results obtained from the MTT assay for cytotoxic activity. In light of these factors, it is not surprising that a linear correlation is often not observed between intrinsic TOP1-targeting activity and cytotoxicity as measured in this assay.

In the case of RPMI8402, a mutant form of TOP1 has been attributed to camptothecin resistance in the variant cell line, CPT-K5.[20] The lack of expression of topoisomerase in P388/CPT45 has been associated with its resistance to camptothecin relative to its parent cell line P388.[21] Cross-resistance to these cell lines by a cytotoxic agent is indicative of TOP1 as a principal target associated with cytotoxic activity. All of the 11*H*-isoquino[4,3-*c*]cinnolin-12-ones evaluated in Table 1 were significantly less cytotoxic toward the camptothecin-resistant variants, CPT-K5 and P388/CPT45, indicating that TOP1 is the principle mechanism associated with their cytotoxic activity.

The cytotoxic activities of **6a**, **6b**, **7a**, **7b**, and **9**–**12** were also assessed in KB3-1 and its two variants KBV-1 and KBH5.0 (Table 2). The over expression of the efflux transporters MDR1 (p-glycoprotein) and BCRP has been observed in KBV-1 and KBH5.0, respectively.[14,22] The over expression of these efflux transporters has been associated with multidrug resistance. Approximately an eightfold difference in cytotoxicity between the variant cells KBV-1 and KBH5.0 relative to KB3-1 is viewed as indicative of a compound being a substrate for one or both of these efflux transporters. In contrast to its *N*-benzyl derivative, **7a** had IC_{50} values in the variant cell lines, KBV-1 and KBH5.0 that clearly indicate that it was a substrate for MDR1 and BCRP. Based upon these criteria, **7b, 11**, and **12** are also substrates for MDR1. Compounds **7a**, **9**, **11**, and **12** also appear as significant substrates for the efflux transporter BCRP. Based upon these comparative cytotoxicity data, the N-benzyl derivatives **6a** and **6b** are not good substrates for either MDR1 or BCRP.

A preliminary assessment of the efficacy of **7a** and **7b** as antitumor agents by both intraperitoneal and oral administration was performed in athymic nude mice. In these studies, MDA-MB-435 human tumor cells were implanted subcutaneously into the right flank of female athymic nude mice. Irinotecan (CPT-11) served as the positive control in this experiment. The results of this bioassay are summarized in Table 3 and are illustrated in Figs. 2 and 3. Throughout this bioassay an effort was made to employ the maxi-

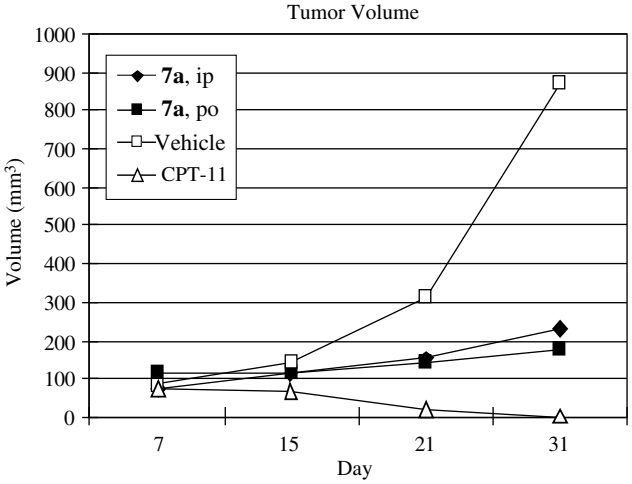


Figure 2. Evaluation of antitumor activity of **7a** and CPT-11.

Table 3
Antitumor activity in athymic nude mice with the human tumor xenograft MDA-MB-435

Compound	# Of mice	Route	Average tumor volume (mm^3)				Total dose (mg/kg)	Total dose (per mouse)[i]
			Day 7	Day 14	Day 21	Day 31		
7a	7	I.P.[a]	146	129	109	104	32.5	0.65
7a	7	P.O.[b]	116	113	144	177	72.5	1.45
7b	6[c]	I.P.[d]	72	112	150	231	14.6	0.29
7b	4[e]	P.O.[f]	82	92	185	253	29.3	0.59
Vehicle	7	I.P.[g]	84	143	313	871	–	–
CPT-11	7	I.P.[h]	75	63	19	2	450	9.0 mg

[a] Initial dose was 2.0 mg/kg qd × 5/week for 2 weeks. Administration was adjusted to 1.0 mg/kg qd × 5/week for two and a half weeks in view of weight loss.
[b] Initial dose was 3.0 mg/kg qd × 5/week for 2 weeks. Administration was adjusted to 4.0 mg/kg qd × 5/week for 1 week and was adjusted back to 3.0 mg/kg qd × 5/week.
[c] One of seven mice died within the 1st week of treatment.
[d] Initial dose was 1.0 mg/kg qd × 4/week. Administration was adjusted to 0.5 mg/kg qd × 5/week for 2 weeks and was adjusted to 0.75 mg/kg qd × 5/week.
[e] Three of seven mice died after 1st week of treatment.
[f] Initial dose was 2.0 mg/kg qd × 4/week. Administration was adjusted to 1.0 mg/kg qd × 5/week for 2 weeks and was adjusted again to 1.5 mg/kg qd × 5/week.
[g] Vehicle consisted of 0.1% citrate in H_2O.
[h] Administration at the dose of 20 mg/kg qd × 5/week.
[i] Assumes an average body weight of 20 g.

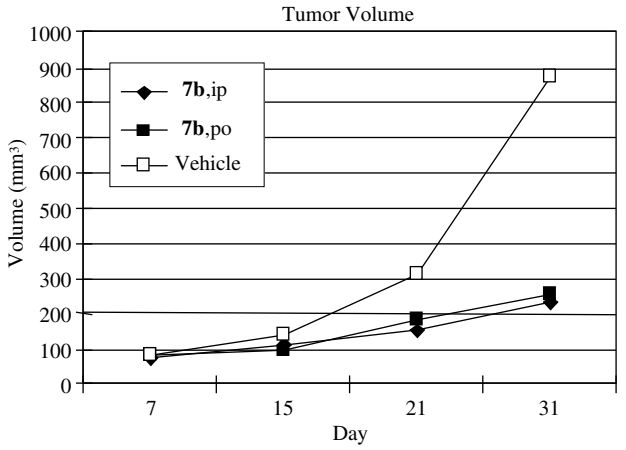


Figure 3. Evaluation of antitumor activity of **7b**.

mal dose of either of these compounds that could be tolerated by the mice. Dose-limiting toxicity as indicated by severe weight loss (>10%) prevented the use of higher doses. In the case of **7b** administered orally, unexpected mortality resulted in the loss of 3 of 7 mice early in the study. As these data indicate, both compounds have potent antitumor activity in vivo, but failed to produce similar tumor regression to that observed with irinotecan. As was observed with **2** in our earlier studies, the dose-limiting toxicity associated with the 11*H*-isoquino[4,3-*c*]cinnolin-12-ones evaluated to date appears to limit their efficacy in vivo. Similar to previous observations with ARC-111, these 11-aza analogs are active when administered by either the oral or the parenteral route. In the case of **7a** and **7b**, it is also evident that, at doses that reflect their maximal tolerated dose, either route of administration achieves similar antitumor activity. The results indicate that similar toxicity and antitumor efficacy are observed at oral doses that are approximately twice that observed for parenteral administration and suggest that both **7a** and **7b** are reasonably well absorbed and are not extensively affected by first-pass metabolism.

These data indicate that **7a** has similar potency to ARC-31, while **7b** appears to be slightly more potent. There was observed minimal differences in efficacy for these analogs relative to what had been previously observed for ARC-31.[17] No clear differences in therapeutic index could be established from these bioassay data. As had been observed with ARC-31, it was evident that equivalent efficacies could be achieved for **7a** as well as **7b** by either oral or intraperitoneal administration. These data indicate that with modest adjustment of dosage, equivalent efficacy can be achieved by either route of administration. For advancing the development of this class of novel TOP1-targeting agents, new analogs are required wherein the dose-limiting toxicity of highly active 11*H*-isoquinoline[4,3-*c*]cinnolin-12-ones is uncoupled to a greater extent from their antitumor activity providing a wider therapeutic window.

3. Conclusions

Several analogs of ARC-31 exhibited very potent TOP1-targeting activity and were highly cytotoxic. Compounds **7a** and **7b** were among the more potent compounds evaluated as TOP1-targeting agents and also exhibited the greater cytotoxic activity (IC_{50} ranging from 0.7 to 0.5 nM, respectively, against KB3-1 cells. Despite their enhanced in vitro activities relative to ARC-31, neither compound proved to be clearly more efficacious than ARC-31 when administered ip or po nor was there any indication of these analogs having an improved therapeutic index. Further studies are needed to identify specific compounds that can offer a clinical advantage by having a wider therapeutic window than either ARC-31 or ARC-111.

4. Experimental

Melting points were determined with Meltemp capillary melting point apparatus. Column chromatography refers to flash chromatography conducted on SiliTech 32–63 mm, (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 as indicated with chemical shifts reported in δ units and tetramethylsilane (TMS) as the internal standard. Coupling constants were 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. Methods for the preparation of **1** and **2** have been previously reported.[13,16] Compound **3** was prepared from 6,7-methylenedioxy-4-cinnolone as previously described.[17]

4.1. General procedure for the formation of 4-amino-6,7-methylenedioxycinnolines (4a and 4b)

The appropriate primary amine (2.0 mL/mmol of **3**) is added with stirring to intermediate **3** followed by copper powder (1.0 equiv). The reaction was then allowed to stir at 100 °C for 5 h. The reaction mixture was then concentrated under reduced pressure, and 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 (MgSO4), filtered, and concentrated. The crude product was purified by flash chromatography eluting with chloroform:methanol.

4.1.1. N^1-(6,7-Methylenedioxycinnolin-4-yl)-N^2-benzyl-N^2-methylethane-1,2-diamine (4a)

Prepared from **3** (2 g, 9.6 mmol) in 92% yield; mp 193–195 °C; IR (neat) 3221; ^{1}H NMR ($CDCl_3$) δ 8.45 (s, 1H), 7.53 (s, 1H), 7.35 (m, 5H), 6.90 (s, 1H), 6.14 (s, 2H), 5.45 (br s, 1H), 3.59 (s, 2H), 3.36 (q, 2H, J = 5.2), 2.77 (t, 2H, J = 5.8), 2.33 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 38.5, 41.1, 53.7, 61.4, 93.5, 101.1, 104.3, 111.7, 126.6, 127.7, 127.8, 128.1, 137.7, 138.7, 146.8, 148.6, 149.7; HRMS (M^++Li) Calcd for $C_{19}H_{20}N_4O_2Li$: 343.1746, found: 343.1760.

4.1.2. N^1-(6,7-Methylenedioxycinnolin-4-yl)-N^2-benzyl-N^2-isopropylethane-1,2-diamine (4b)

Prepared from **3** (2 g, 9.6 mmol) in 75% yield; mp 162–164 °C; IR (neat) 3246; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H), 7.46 (s, 1H), 7.24 (m, 5H), 6.71 (s, 1H), 6.09 (s, 2H), 5.39 (br s, 1H), 3.53 (s, 2H), 3.12 (m, 2H), 3.00 (m, 1H), 2.80 (t, 2H, J = 5.6), 1.05 (d, 6H, J = 6.6); ^{13}C NMR ($CDCl_3$) δ 17.2, 38.7, 46.5, 48.8, 52.6, 93.6, 101.1, 104.2, 112.0, 126.4, 127.7, 127.8, 137.8, 138.9, 148.5, 149.7; HRMS (M^++Li) Calcd for $C_{21}H_{24}N_4O_2Li$: 371.2059, found: 371.2063.

4.2. General procedure for the formation of 2-Iodo-4,5-dimethoxybenzamides (5a and 5b)

Oxalyl chloride (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 under reduced pressure. To the residue was added a solution of the appropriate 4-amino-6,7-methylenedioxycinnoline (1.0 equiv), triethylamine (2.0 equiv) in CH_2Cl_2

(60 mL per 4 mmol aminocinnoline). The reaction mixture was then stirred at reflux under N_2 for 5 h. The reaction mixture was cooled and washed with satd. $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The product was isolated using flash chromatography eluting with chloroform:methanol.

4.2.1. *N*-(6,7-Methylenedioxycinnnolin-4-yl)-*N*-[2-(benzyl(methyl)amino)ethyl]-2-iodo-4,5-dimethoxybenzamide (5a)

Prepared from **4a** (2.5 g, 7.44 mmol) as a brown sticky glue in 85% yield; IR (neat) 1655; ^{1}H NMR ($CDCl_3$) δ 9.08 (s, 1H), 7.63 (s, 1H), 7.33 (s, 1H), 7.18 (m, 5H), 6.98 (s, 1H), 6.36 (s, 1H), 6.17 (s, 2H), 4.42 (m, 1H), 3.69 (s, 3H), 3.62 (m, 1H), 3.48 (s, 2H), 3.38 (s, 3H), 2.75 (q, 2H, J = 4.8), 2.13 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 41.6, 46.2, 54.4, 54.7, 55.1, 61.6, 95.7, 101.9, 104.5, 109.6, 120.9, 122.0, 126.2, 127.3, 127.5, 128.1, 131.9, 135.1, 137.4, 143.8, 147.2, 149.0, 150.0, 150.7, 151.4, 168.9; HRMS (M^++H) Calcd for $C_{28}H_{27}IN_4O_5H$: 627.1104, found: 627.1037.

4.2.2. *N*-(6,7-Methylenedioxycinnnolin-4-yl)-*N*-[2-(benzyl(isopropyl)amino)ethyl]-2-iodo-4,5-dimethoxybenzamide (5b)

Prepared from **4b** (2.56 g, 7.05 mmol) as a brown sticky glue in 52% yield; IR (neat) 1659; ^{1}H NMR ($CDCl_3$) δ 9.08 (s, 1H), 7.83 (s, 1H), 7.48 (s, 1H), 7.47 (s, 1H), 7.29 (s, 1H), 7.19 (m, 5H), 6.39 (s, 2H), 4.45 (m, 1H), 3.89 (s, 3H), 3.69 (s, 2H), 3.52 (s, 3H), 3.41 (m, 1H), 3.18 (m, 3H), 1.24 (d, 3H, J = 7.0), 1.21 (d, 3H, J = 6.8); ^{13}C NMR ($CDCl_3$) δ 17.4, 48.0, 49.1, 51.6, 54.7, 55.0, 55.4, 95.8, 102.1, 104.9, 109.9, 119.5, 121.3, 121.6, 126.1, 127.4, 128.2, 128.7, 132.0, 135.4, 143.8, 147.5, 149.4, 150.2, 151.0, 151.5, 169.0; HRMS (M^++H) Calcd for $C_{30}H_{31}IN_4O_5H$: 655.1417, found: 655.1399.

4.3. General procedure for the preparation of 11-substituted 11*H*-isoquino[4,3-*c*]cinnolin-12-ones via cyclization of *o*-iodobenzamides (6a and 6b)

A mixture of the 4-amino-6,7-methylenedioxycinnoline-*o*-iodobenzamide derivative (1.0 equiv), $Pd(OAc)_2$ (0.2 equiv), P(*o*-tolyl)$_3$ (0.4 equiv), and Ag_2CO_3 (2.0 equiv) was heated to reflux in DMF (30 mL per equiv) under nitrogen atmosphere with stirring for 30 min. 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 under reduced pressure, and the residue chromatographed on silica gel using chloroform: methanol.

4.3.1. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-benzyl(methyl)amino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (6a)

Prepared from **5a** (1.5 g, 2.4 mmol) in 29% yield; mp 245–247 °C; IR (neat) 1657; ^{1}H NMR ($CDCl_3$) δ 8.56 (s, 1H), 8.02 (s, 1H), 7.77 (s, 1H), 7.72 (s, 1H), 7.23 (m, 5H), 6.24 (s, 2H), 4.61 (t, 2H, J = 6.6), 4.13 (s, 3H), 4.04 (s, 3H), 3.57 (s, 2H), 3.07 (t, 2H, J = 6.6), 2.30 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 41.6, 46.6, 54.7, 55.4, 55.7, 62.3, 98.6, 101.7, 103.2, 105.4, 106.8, 112.6, 118.5, 126.2, 127.3, 128.0, 128.1, 129.9, 133.2, 137.6, 148.3, 149.1, 150.2, 150.4, 153.3, 161.9; HRMS (M^++H) Calcd for $C_{28}H_{26}N_4O_5H$: 499.1981, found: 499.1950.

4.3.2. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-benzyl(isopropyl)amino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (6b)

Prepared from **5b** (2.4 g, 3.67 mmol) in 9% yield; mp 235–237 °C; IR (neat) 1653; ^{1}H NMR ($CDCl_3$) δ 8.57 (s, 1H), 7.96 (s, 1H), 7.77 (s, 1H), 7. 72 (s, 1H), 7.15 (m, 5H), 6.24 (s, 2H), 4.48 (t, 2H, J = 6.2), 4.13 (s, 3H), 4.04 (s, 3H), 3.65 (s, 2H), 3.12 (t, 2H, J = 6.2), 2.94 (m, 1H), 1.00 (d, 6H, J = 6.6); ^{13}C NMR ($CDCl_3$) δ 16.8, 47.4, 47.6, 50.0, 53.9, 55.4, 55.6, 98.7, 101.7, 103.2, 105.4, 106.9, 112.6, 118.6, 125.8, 127.2, 127.5, 128.0, 130.0, 133.3, 139.3, 148.2, 149.1, 150.0, 150.3, 153.2, 162.0; HRMS (M^++H) Calcd for $C_{30}H_{30}N_4O_5H$: 527.2294, found: 527.2260.

4.4. General procedure for the syntheses of 7a and 7b

To a solution of *N*-benzylated cinnoline derivative (**6a** or **6b**) in acetic acid (40 mL) and formic acid (10 mL) was added Pd black (150 mg) and stirred at room temperature for 45 min. Reaction mixture was concentrated under reduced pressure, basified with 10% NaOH and extracted with chloroform. The organic layer was dried over Na_2SO_4, filtered, and concentrated. Crude was purified by flash chromatography eluting with chloroform: methanol.

4.4.1. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-methylamino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (7a)

Prepared from **6a** (200 mg, 0.4 mmol) in 73% yield; mp 245 °C (decomp.); IR (neat) 3421, 1653; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 8.55 (s, 1H), 7.77 (s, 1H), 7.74 (s, 1H), 7.69 (s, 1H), 6.21 (s, 2H), 4.56 (t, 2H, J = 6.6), 4.12 (s, 3H), 4.03 (s, 3H), 3.26 (t, 2H, J = 6.6), 2.50 (s, 3H); ^{13}C NMR ($CDCl_3$+ CD_3OD) δ 35.3, 47.6, 49.8, 55.4, 55.7, 97.8, 101.9, 103.2, 105.3, 106.9, 112.7, 118.5, 128.0, 130.0, 133.4, 137.6, 148.3, 149.4, 150.3, 150.5, 153.5, 162.4; HRMS (M^++H) Calcd for $C_{21}H_{20}N_4O_5H$: 409.1512, found: 409.1531.

4.4.2. 2,3-Dimethoxy-8,9-methylenedioxy-11-[(2-isopropylamino)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (7b)

Prepared from **6b** (150 mg, 0.285 mmol) in 82% yield; mp 267 °C (dec); IR (neat) 3444, 1652; ^{1}H NMR ($CDCl_3$) δ 8.57 (s, 1H), 7.90 (s, 1H), 7.78 (s, 1H), 7. 73 (s, 1H), 6.21 (s, 2H), 4.52 (t, 2H, J = 6.6), 4.14 (s, 3H), 4.05 (s, 3H), 3.34 (t, 2H, J = 6.6), 2.97 (m, 1H), 1.12 (d, 6H, J = 6.2); ^{13}C NMR ($CDCl_3$) δ 22.7, 45.0, 47.9, 48.1, 55.4, 55.7, 98.4, 101.8, 103.3, 105.5, 106.8, 112.6, 118.5, 128.1, 130.0, 133.2, 148.3, 149.2, 150.2, 150.4, 153.3, 162.1; HRMS (M^++H) Calcd for $C_{23}H_{24}N_4O_5H$: 437.1825, found: 437.1830.

4.5. 2,3-Dimethoxy-8,9-methylenedioxy-11*H*-isoquino[4,3-*c*]cinnolin-12-one-11-[2-*N*,*N*,*N*,-trimethyethanaminium]iodide (8)

To a solution of **ARC-31** (100 mg, 0.237 mmol) in a mixture of DCM and methanol (20 mL, 4:1) was added methyl iodide (0.145 mL, 2.3 mmol) drop wise at room temperature and stirred overnight. Reaction mixture was concentrated under reduced pressure and dried under high vacuum to give **8** in quantitative yield; mp 255 °C (dec); IR (neat) 1461; ^{1}H NMR (DMSO-d_6) δ 8.38 (s, 1H), 7.82 (s, 1H), 7.69 (s, 1H), 7. 67 (s, 1H), 6.40 (s, 2H), 4.96 (t, 2H, J = 6.2), 4.04 (s, 3H), 3.95 (s, 3H), 3.79 (t, 2H, J = 6.2), 3.19 (s, 9H); ^{13}C NMR (DMSO-d_6) δ 54.7, 55.6, 55.8, 61.0, 98.1, 103.2, 105.0, 107.5, 112.6, 118.4, 127.9, 129.6, 133.6, 148.6, 150.4, 150.8, 151.1, 153.9, 161.7; HRMS (M^++H) Calcd for $C_{23}H_{24}IN_4O_5H$: 437.1825, found: 437.1805.

4.6. 2,3-Dimethoxy-8,9-methylenedioxy-11-[2-(1,3-dihydroxy-2-(hydroxymethyl)propan-2-ylamino)ethyl]-1*H*-isoquino[4,3-*c*]cinnolin-12-one (9)

To a solution of **8** (113 mg, 0.2 mmol) in anhydrous DMSO (6 mL) in a sealed tube was added tris(hydroxymethyl)aminomethane (242 mg, 2.0 mmol) and stirred at 150 °C for 4 h. DMSO was removed by Kugelrohr distillation, and the crude was purified by flash chromatography eluting with chloroform:methanol to give compounds **9** and **10**.

Yield of **9**; 11%; mp 245–247 °C; IR (neat) 3366, 1654; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 8.56 (s, 1H), 8.09 (s, 1H, -NH), 7.77 (s, 1H), 7.76 (s, 1H), 7. 75 (s, 1H), 6.25 (s, 2H), 4.53 (t, 2H, J = 5.4), 4.19 (s, 3H, —OH), 4.16 (s, 3H), 4.07 (s, 3H), 3.62 (s, 6H), 3.53 (t, 2H, J = 5.4); ^{13}C NMR (DMSO-d_6) δ 59.9, 60.1, 64.5, 65.3, 67.3, 72.2, 103.8, 107.4, 109.2, 111.6, 116.7, 122.8, 132.1, 134.5, 137.2, 152.8, 154.3, 155.0, 155.0, 157.8, 165.9; HRMS (M^++H) Calcd for $C_{24}H_{26}N_4O_8H$: 499.1829, found: 499.1818.

4.7. 2,3-Dimethoxy-8,9-methylenedioxy-11-(2-hydroxyethyl)-11*H*-isoquino[4,3-*c*]cinnolin-12-one (10)

Yield of **10**; 6%; mp 274 °C (dec); IR (neat) 3415, 1658; ^{1}H NMR ($CDCl_3$+CD_3OD) δ 8.60 (s, 1H), 7.82 (s, 1H), 7.79 (s, 1H), 7. 71 (s, 1H), 6.26 (s, 2H), 4.63 (t, 2H, J = 5.4), 4.34 (t, 2H, J = 5.4), 4.17 (s, 3H), 4.08 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 52.3, 55.6, 55.8, 61.0, 98.1, 103.2, 105.0, 107.5, 112.6, 118.4, 127.9, 129.6, 133.6, 148.6, 150.4, 150.8, 151.1, 153.9, 161.7; HRMS (M^++H) Calcd for $C_{20}H_{17}N_3O_6H$: 396.1196, found: 396.1189.

4.8. 2,3-Dimethoxy-8,9-methylenedioxy-11-(2-[1*H*-imidazo-1-yl)ethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (11)

To a solution of **8** (100 mg, 0.18 mmol) in anhydrous DMSO (5 mL) in a sealed tube was added imidazole (120 mg, 1.8 mmol) and stirred at 150 °C for 4 h. DMSO was removed by Kugelrohr distillation, and the crude product was purified by flash chromatography eluting with chloroform:methanol to give compound **11** in 29% and a 10% yield of **10**. Compound **11** had mp 280–282 °C (dec); IR (neat) 1646; ^{1}H NMR (CD_3COOD) δ 8.69 (s, 1H), 8.20 (s, 1H), 7.72 (s, 1H), 7.60 (s, 1H), 7. 36 (s, 1H), 7.15 (s, 1H), 7.13 (s, 1H), 6.12 (s, 2H), 5.01 (br s, 2H), 4.66 (br s, 2H), 3.89 (s, 3H), 3.81 (s, 3H); HRMS (M^++H) Calcd for $C_{23}H_{19}N_5O_5H$: 446.1464; found: 446.1455.

4.9. 2,3-Dimethoxy-8,9-methylenedioxy-11-[2-((2-*N*,*N*-dimethylaminoethyl)methylaminoethyl]-11*H*-isoquino[4,3-*c*]cinnolin-12-one (12)

To a solution of **8** (100 mg, 0.18 mmol) in anhydrous DMSO (5 mL) in a sealed tube was added *N*,*N*,*N*′-trimethylethylenediamine (237 mg, 2.3 mmol) and stirred at 150 °C for 1 h. DMSO was removed by Kugelrohr distillation and the crude product was purified by flash chromatography eluting with chloroform:methanol to give compound **12** in 10% and **10** in 12% yield, mp 218–220 °C; IR ($CHCl_3$) 1652; ^{1}H NMR ($CDCl_3$) δ 8.63 (s, 1 H), 8.04 (s, 1H), 7.83 (s, 1H), 7.81 (s, 1H), 6.23 (s, 2H), 4.66 (t, 2H, J = 5.4), 4.15 (s, 3H), 4.06 (s, 3H), 3.09 (t, 2H, J = 5.4), 2.58 (m, 2H), 2.37 (m, 5H), 2.20 (s, 6H); HRMS (M^++H) Calcd for $C_{25}H_{28}N_5O_5H$: 480.2241; found: 480.2240.

4.10. 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. Plasmid YepG was also purified by the alkali lysis method followed by phenol deproteination and CsCl/ethidium isopycnic centrifugation method as described.[23] 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.[24] The cleavage assays were performed as previously reported.[25,26] 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). The REC value reflects the concentrations relative to camptothecin, whose value is arbitrarily assumed as 0.2, that is able to produce the same 10% cleavage on the plasmid DNA in the presence of human topoisomerase I.

4.11. Cytotoxicity assays

The cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA). The human lymphoblast RPMI 8402 and its camptothecin-resistant variant cell line, CPT-K5 was provided by Dr. Toshiwo Andoh (Aichi Cancer Center Research Institute, Nagoya, Japan).[20] The P388 mouse leukemia cell line and its CPT-resistant TOP1-deficient variant P388/CPT45[21] were obtained from Michael R. Mattern and Randal K. Johnson (Glaxo-SmithKline, King of Prussia, PA). The KB3-1 cell line and its multidrug-resistant variant KBV-1[22] were obtained from K.V. Chin (The Cancer Institute of New Jersey, New Brunswick, NJ). The KBH5.0 cell line as noted previously[14] was derived from KB3-1 by stepwise selection against Hoechst 33342. 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). For determination of IC_{50}, cells were exposed continuously for FOUR 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.

4.12. Human tumor xenograft

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 4 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 arrival within the animal facility, 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 x 5/8"). The MDA-MB-435 cells were grown in 75 cm^2 flasks using RPMI 1640 Media and 10% fetal bovine serum. Irinotecan (CPT-11) was used as the positive control. Tumors were of sufficient size at 19–20 days after inoculation. Tumor-bearing mice were evenly matched in each experimental group based on tumor volume. 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 $(l^* w^2)/2$. Every mouse in this study was weighed individually on a daily basis. Dose adjustments for each experimental group, as indicated in Table 3, were made throughout the study based upon the effect or lack of an effect of treatment on average body weights. Tumor volume was determined for each individual mouse every other day.

Acknowledgments

This study was supported by National Cancer Institute Grants CA098127 (E.J.L) and CA39662 (L.F.L.) and Genzyme Corporation.

Mass spectrometry was provided by Washington University Resource for Biomedical and Bio-organic Spectrometry with support from the NIH National Center for Research Resources (Grant No. P41RR0954).

References

1. Zhang, H.; Wang, J. C.; Liu, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 1060.
2. Liu, L. F. *Annu. Rev. Biochem.* **1989**, *58*, 351.
3. Chen, A. Y.; Liu, L. F. *Annu. Rev. Pharmacol. Toxicol.* **1994**, *34*, 191.
4. Pommier, Y.; Kohlhagen, G.; Laco, G. S.; Kroth, H.; Sayer, J. M.; Jerina, D. M. *J. Biol. Chem.* **2002**, *277*, 13666.
5. Wall, J. G.; Burris, H. A.; Von-Hoff, D. D.; Rodriquez, G.; Kneuper-Hall, R.; Shaffer, D.; O'Rourke, T.; Brown, T.; Weiss, G.; Clark, G.; McVea, S.; Brown, J.; Johnson, R.; Friedman, C.; Smith, B.; Mann, W. S. *Anticancer Drugs* **1992**, *3*, 337.
6. Burke, T. G.; Mi, Z. *J. Med. Chem.* **1993**, *36*, 2580.
7. Burke, T. G.; Mi, Z. *Anal. Biochem.* **1993**, *212*, 285.
8. Chen, A. Y.; Yu, C.; Potmesil, M.; Wall, M. E.; Wani, M. C.; Liu, L. F. *Cancer Res.* **1991**, *51*, 6039.
9. Chu, X.; Kato, Y.; Sugiyama, Y. *Cancer Res.* **1997**, *57*, 1934.
10. Kawabata, S.; Oka, M.; Shiozawa, K.; Tsukamoto, K.; Nakatomi, K.; Soda, H.; Fududa, M.; Ikegami, Y.; Sugahara, K.; Yamada, Y.; Kamihira, S.; Doyle, L. A.; Ross, D. D.; Kohno, S. *Biochem. Biophys. Res. Commun.* **2001**, *280*, 1216.
11. Yang, C. H.; Schneider, E.; Kuo, M. L.; Volk, E. L.; Roccchi, E.; Chen, Y. C. *Biochem. Pharmacol.* **2000**, *60*, 831.
12. Ruchelman, A. L.; Singh, S. K.; Wu, X. H.; Ray, A.; Yang, J.-M; Li, T.-K; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3333.
13. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Li, T.-K.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 2061.
14. Li, T.-K.; Houghton, P. J.; Desai, S. D.; Daroui, P.; Liu, A. A.; Hars, E. S.; Ruchelman, A. L.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **2003**, *63*, 8400.
15. Ruchelman, A. L.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *J. Med. Chem.* **2005**, *48*, 792.
16. Yu, Y.; Singh, S. K.; Liu, A.; Li, T.-K.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2003**, *11*, 1475.
17. Ruchelman, A. L.; Singh, S. K.; Ray, A.; Wu, X.; Yang, J.-M.; Zhou, N.; Liu, A.; Liu, L. F.; LaVoie, E. J. *Bioorg. Med. Chem.* **2004**, *12*, 795.
18. Wadkins, R. M.; Bearss, D.; Manikumar, G.; Mansukhlal, C.; Wani, M. C.; Wall, M. E.; Von Hoff, D. D. *Cancer Res.* **2004**, *64*, 6679.
19. Feng, W.; Satyanarayana, M.; Tsai, T.-C.; Liu, A. A.; Liu, L. F.; LaVoie, E. J. *Bioorg Med. Chem. Lett.* **2008**, *18*, 3570.
20. Andoh, T.; Ishii, K.; Suzuki, Y.; Ikegami, Y.; Kusunoki, Y.; Takemoto, Y.; Okada, K. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5565.
21. Woessner, R. D.; Eng, W. K.; Hofmann, G. A.; Rieman, D. J.; McCabe, F. L.; Hertzberg, R. P.; Mattern, M. R.; Tan, K. B.; Johnson, R. K. *Oncol. Res.* **1992**, *4*, 481.
22. Gervasoni, J. E., Jr.; Fields, S. Z.; Krishna, S.; Baker, M. A.; Rosado, M.; Thuraisamy, K.; Hindenburg, A. A.; Taub, R. N. *Cancer Res.* **1991**, *51*, 4955.
23. Maniatis, T.; Fritsch, E. F.; Sambrook, J. *Molecular Cloning, A Laboratory Manual*; Cold Spring Harbor Laboratory: Cold Spring Harbor, NJ, 1982. pp. 149–185.
24. Tewey, K. M.; Rowe, T. C.; Yang, L.; Hallogan, B. C.; Liu, L. F. *Science* **1984**, *226*, 466.
25. Gatto, B.; Sanders, M. M.; Yu, C.; Wu, H.-Y. ; Makhey, D.; LaVoie, E. J.; Liu, L. F. *Cancer Res.* **1996**, *56*, 2795.
26. Wang, H.; Mao, Y.; Chen, A.; Zhou, N.; LaVoie, E. J.; Liu, L. F. *Biochemistry* **2001**, *40*, 3316.