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Diaza- and Triazachrysenes: Potent Topoisomerase-Targeting Agents with Exceptional Antitumor Activity against the Human Tumor Xenograft, MDA-MB-435

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Abstract—Several 5,12-diazachrysen-6-ones and 5,6,11-triazachrysen-12-ones were synthesized with varied substituents at the 5- or 11-position, respectively. Each compound was evaluated for its potential to stabilize the cleavable complex formed with TOP1 and DNA. Two analogues with very potent TOP1-targeting activity, **3a** and **4a**, exhibited cytotoxic activity with IC_{50} values at or below 2 nM against RPMI8402. Compound **3a** was active in vivo by either ip or po administration in the human tumor xenograft athymic nude mice model.

DNA topology is regulated by DNA topoisomerases that catalyze the breaking and rejoining of DNA strands.[1–3] Topoisomerases are critical to both replication and transcription. There are two major types of topoisomerases, type I [e.g., topoisomerase I (TOP1)] and type II [e.g., topoisomerase II (TOP2)], based upon differences in their initial mechanisms wherein a single- or double-stranded DNA break is implicated.[1–3] Topoisomerase-targeting agents that can stabilize the cleavable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer. This drug-induced stabilization of the enzyme–DNA cleavable complex effectively converts these nuclear enzymes into cellular poisons.

Camptothecin was the first agent identified as a TOP1-targeting agent.[4] The extensive studies on camptothecin and its structurally related analogues have resulted in the clinical development of two TOP1-targeting drugs, topotecan (Hycamptin®) and irinotecan (CPT-11/Camptosar®). Both of these drugs have incorporated within their structure the camptothecin-ring system, which includes the presence of a γ-lactone. Hydrolysis of this lactone moiety 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 resistance have prompted further studies on the development of novel TOP1-targeting agents.[8–10] Recently, bi- and terbenzimidazoles,[11,12] benz[*a*]anthracenes,[13] certain benzo[*c*]phenanthridine and protoberberine alkaloids and their synthetic analogues,[14,15] indolocarbazoles,[16] the fungal metabolites bulgarein,[17] and saintopin,[18] several indenoisoquinolines[19] and benzophenazines[20] have been identified as TOP1-targeting agents.

Studies in our laboratory originating with protoberberine analogues and more recently with non-charged heterocyclic analogues have revealed that appropriately substituted benzo[*i*]phenanthridines and

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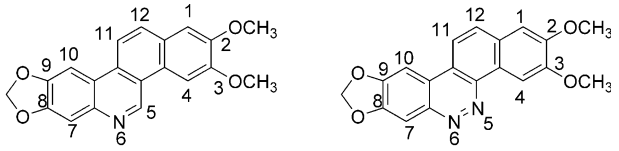
dibenzo[*c,h*]cinnolines can exhibit significant TOP1-targeting activity and cytotoxicity. Structure–activity studies have revealed that the presence of a methylenedioxy group on the benzo-ring fused adjacent to the nitrogen heteroatom is associated with enhanced activity. In addition, the presence of two methoxyl groups on the aromatic ring distal from the methylenedioxy group favors both TOP1-targeting activity and cytotoxicity. These structural characteristics associated with enhanced activity are embodied in the benzo[*i*]phenanthridine, **1**, and the dibenzo[*c,h*]cinnoline, **2**, illustrated in Figure 1.

Further studies on aza-derivatives of **2** demonstrated that the presence of an additional nitrogen heteroatom at the 11- or 12-position did not negatively affect relative potency with regard to either TOP1 targeting activity or cytotoxicity. Because of the poor solubility of both **1** and **2**, difficulties were encountered in developing suitable formulations for assessment of their relative antitumor activity in vivo. Efforts were directed toward the preparation of analogues that would allow for incorporation of functionality to enhance solubility. One suitable modification would be to develop 5,12-diazachrysen-6-ones and 5,6,11-triazachrysen-12-ones that possessed varied substituents at either the 5- or 11-position, respectively. The synthesis and the pharmacological evaluation of these agents are discussed in the present study.



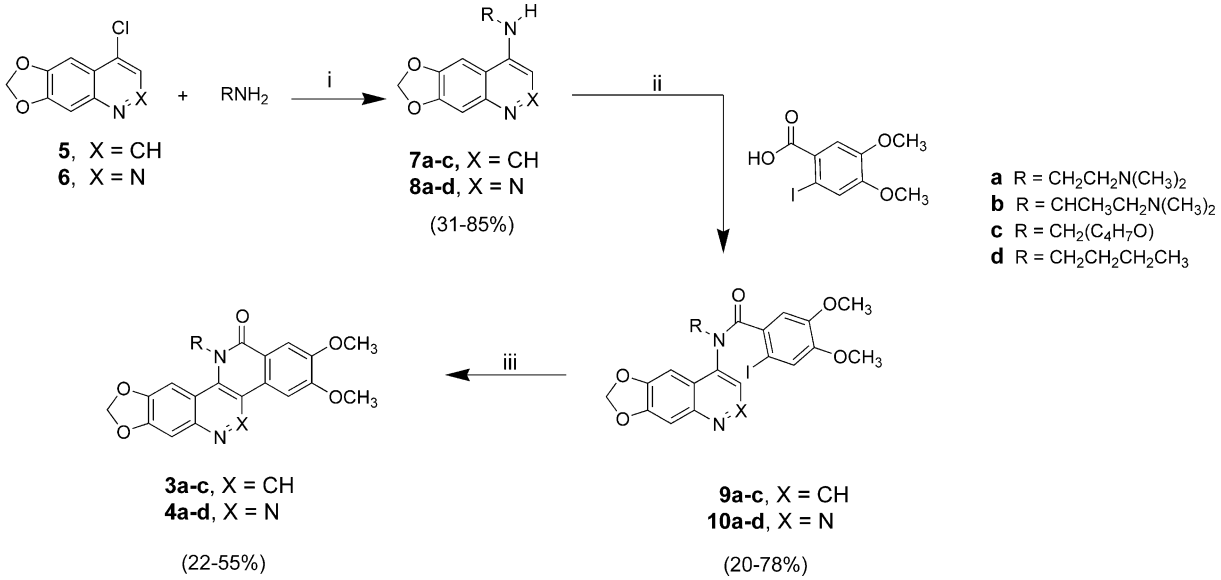
1 **2**

Figure 1. Benzo[*i*]phenanthridine and dibenzo[*c,h*]cinnoline derivatives with TOP1-targeting activity.

Several 5,12-diazachrysen-6-ones, **3a–c**, and 5,6,11-triazachrysen-12-ones, **4a–d**, were selected for synthesis. It was envisioned that a similar approach to that used by Harayama et al. for benzo[*c*]phenanthridine alkaloids could be used for the synthesis of either series of compounds.[21] The synthetic approach that was employed is outlined in Scheme 1. The starting material for compounds **3a–c** and **4a–d** was either 4-chloro-6,7-methylenedioxyquinoline, **5**, or 4-chloro-6,7-methylenedioxycinnoline, **6**. Intermediates **5** and **6** were prepared from 4-hydroxy-6,7-methylenedioxyquinoline[22] and 4-hydroxy-6,7-methylenedioxycinnoline[23] as previously described.[22,24,25] For the formation of the 4-aminoquinoline derivatives, **7a–c**, reactions were performed in the presence of phenol as detailed in the literature.[26,27] The preferred method for the formation of 4-aminocinnolines, **8a–d**, was to use the appropriate primary amine, RNH_2, as solvent.[28,29]

The synthesis of 2-iodo-4,5-dimethoxybenzoic acid was performed as described in the literature.[30] Conversion of this acid to the acid chloride using oxalyl chloride, followed by immediate reaction with the appropriate 4-(alkylamino)-6,7-methylenedioxyquinoline or 4-(alkylamino)-6,7-methylenedioxycinnoline provided variable yields of the amides, **9a–c** and **10a–d**. The Heck reaction was employed for the cyclization of the *o*-iodobenzamides to provide the 5,12-diazachrysen-6-ones, **3a–c**, as well as the 5,6,11-triazachrysen-12-ones, **4a–d**.[31]

Compounds **3a–c** and **4a–d** were evaluated for the ability to induce DNA cleavage in the presence of TOP1 and TOP2. The results of these analyses are listed in Table 1. The influence of structure differences on TOP1-targeting activity is evident from these data. Enhanced intrinsic TOP1-targeting activity is observed with **4a** relative to **3a**. This trend is consistent among each pair of similarly substituted diazachysen-6-one and triazachysen-12-one. Thus, the relative potency of **4a** > **3a**, **4b** > **3b**, and **4c** > **3c**. These data are consistent with data obtained with benzo[*i*]phenanthridines and diben-



Scheme 1. (i) **5**: Reflux in C_6H_5OH for 2.5 h, then add RNH_2 at 100 °C; **6**: RNH_2, Cu powder, reflux; (ii) $(COCl)_2$, anhyd CH_2Cl_2 and TEA, reflux; (iii) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, in DMF, reflux.

Table 1. TOP1-targeting activity and cytotoxicity of 5,12-diazachrysen-6-ones, **3a–c**, and 5,6,11-triazachrysen-12-ones, **4a–d**

Compd	TOP1-mediated DNA cleavage[a]	Cytotoxicity IC_{50} (μM)	
		RPMI8402[b]	CPT-K5[c]
3a	0.5	0.002	0.90
3b	>1000	0.57	3.4
3c	200	0.15	>10
4a	0.3	0.001	0.60
4b	1000	0.26	3.6
4c	30	0.010	>10
4d	1.0	0.004	10
CPT-11	25	0.57	>10
Topotecan	1.0	0.012	>10
VM-26	>1000	0.22	0.28

[a]Topoisomerase I cleavage values are reported as REC, relative effective concentration, that is concentrations relative to topotecan, whose value is arbitrarily assumed as 1, that are able to produce the same cleavage on the plasmid DNA in the presence of human topoisomerase I. TOP2-mediated cleavage was >100 relative to VM26 for all compounds listed.
[b]RPMI8402 is a human lymphoblast tumor cell line.
[c]CPT-K5 is a camptothecin-resistant variant of RPMI8402 that possesses a functional, but mutant TOP1.

zo[*c,h*]cinnolines. In these studies, dibenzo[*c,h*]cinnolines proved to be significantly more potent in inducing DNA cleavage in the presence of TOP1 than similarly substituted benzo[*i*]phenanthridines. The relative potency of the 5,12-diazachrysen-6-ones with respect to inducing DNA fragmentation in the presence of TOP1 is **3a** > **3c** >> **3b**. Each of the varied 5-substituents in **3a–c** had a similar effect on activity among the triazachrysen-12-ones. The relative TOP1-targeting activity of the 5,6,11-triazachrysen-12-ones is **4a** > **4d** > **4c** >> **4b**.

While the *n*-butyl analogue **4d** is slightly less active than **4a**, the retention of potent TOP1-targeting activity by this derivative suggests that the 2-(*N*,*N*-dimethylaminoethyl) group of compound **4a** is not a critical requirement for potent activity among these 5,6,11-triazachrysen-12-ones. The effect of the β-methyl substituent in the case of both **3b** and **4b** is dramatic. Clearly, the presence of this substituent had a decisively negative impact on the ability of these compounds to effectively stabilize cleavable complex formation in the presence of enzyme and DNA. None of these compounds exhibited induced DNA cleavage in the presence of TOP2 (Fig. 2).

The presence of an alkyl substituent at N5 of 5,12-diazachrysen-6-ones or N11 of 5,6,11-triazachrysen-12-ones was shown in modeling studies to result in a twist within these pentacyclic compounds. The lack of planarity associated with **3a–c** and **4a–d** could, in part, be responsible for an absence of activity observed with TOP2. For example for **3a–c**, models, geometry optimized at the AM1 level using Spartan (Wavefunction, Inc.) suggest that there exists an approximately 14–22° torsion angle involving C4,C4a,C4b,N5.[32] Previous studies on coralyne and benzo[*i*]phenanthridine derivatives have indicated that non-planar analogues generally are not as effective in stabilizing the cleavable complex with TOP2.

The cytotoxicity data obtained using the human lymphoblastoma cell line, RPMI8402, and its camptothecin-resistant variant, CPT-K5 are consistent with the relative intrinsic potency of these compounds as TOP1-targeting agents. The basis for the resistance of CPT-K5 cells to camptothecin is related to a mutant, but functional form of TOP1. In contrast to results observed with some benzo[*i*]phenanthridine derivatives, all of the compounds in the present study exhibited significant cross-resistance in the CPT-K5 cell line.

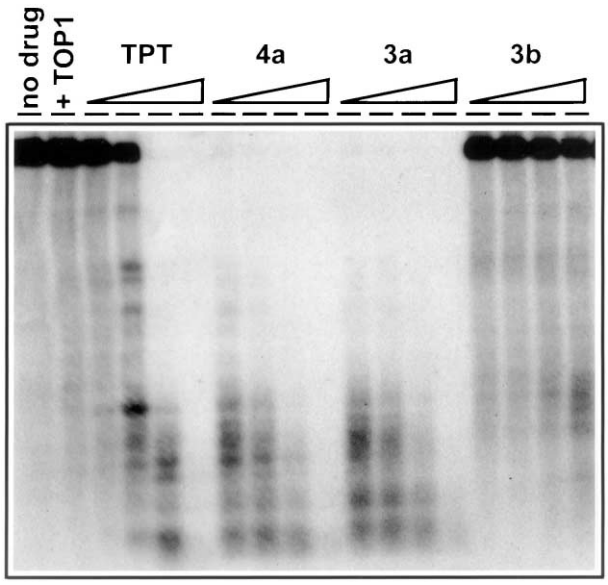


Figure 2. Stimulation of enzyme-mediated DNA cleavage by **4a**, **3a**, **3b** and topotecan (TPT) using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.01 to 10 μM.

Compounds **3a**,**b** and **4a**,**b** were found to have excellent solubility in chloroform and to have water solubility >2.0 mg/mL as their citrate salts. Despite the fact that substituents at the 11-position do disrupt the planarity of these diazachrysene and triazachrysene derivatives, compounds **3c** and **4c**,**d** had limited solubility and were difficult to formulate for in vivo studies. Thus, compounds **3a** and **4a** were selected for the initial in vivo assessment of the efficacy of these novel TOP1- targeting agents. NCR-*nude-nude* mice obtained from Taconic Farms (Albany, NY, USA) were inoculated with $1.2–1.5 \times 10^6$ MDA-MB-435 cells. Mice were administered 2.0 mg/kg of **3a** po or ip. Mice treated with **4a** received 1.0 mg/kg ip and po per injection. Mice treated with **3a** or **4a** received doses ip or po as tolerated, followed by periods of 1–3 days of no treatment. The total average dose administered per mouse is provided in Table 2. Irinotecan (CPT-11) was administered ip for 5 consecutive days per week at a dose of 25 mg/kg. Treatment of all groups was continued for 31 days. The results of this assay are summarized in Table 2.

These data demonstrate that select 5,12-diazachrysen-6-ones and 5,6,11-triazachrysen-12-ones are potent TOP1-targeting agents with exceptional cytotoxic activity. Based upon the studies performed with **3a**, 5,12-diazachrysen-6-one derivatives have the potential to exhibit exceptional potency in vivo as antitumor agents by either parenteral or oral administration.

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

Compd	Route	Average tumor volume (mm^3)				Average total dose (mg/mouse)
		Day 7	Day 13	Day 21	Day 31	
3a	po[a]	159	148	99	62	0.78
3a	ip[a]	123	84	65	40	0.73
4a	po[a]	154	183	207	200	0.27
4a	ip[a]	167	211	252	403	0.22
CPT-11	ip[b]	118	116	36	11	13.88
Vehicle[c]	ip[b]	199	234	356	472	

[a]Seven mice per group.
[b]Six mice per group.
[c]Vehicle controls consisted of 0.01% citrate administered 5 days per week.

Acknowledgements

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31. Diazachrysene and triazachrysene derivatives prepared were purified using flash chromatography using silica gel. Each compound gave appropriate HRMS data and provided ^{1}H NMR spectra in agreement with assigned structures. ^{1}H NMR data for **3a**–**c** and **4a**–**d** are given (Varian Gemini 200 MHz spectrometer, δ in ppm, in $CDCl_3$ with TMS as internal standard): (**3a**) 2.33 (s, 6H), 3.04 (t, 2H, $J=7.2$ Hz), 4.07 (s, 3H), 4.14 (s, 3H), 4.64 (t, 2H, $J=7.2$ Hz), 6.18 (s, 2H), 7.47 (s, 1H), 7.68 (s, 1H), 7.89 (s, 2H), 9.37 (s, 1H); (**3b**) 1.95–1.98 (m, 9H), 2.77 (dd, 1H, $J=12.0$, 8.0 Hz), 3.21 (dd, 1H, $J=12.0$, 8.0 Hz), 4.06 (s, 3H), 4.13 (s, 3H), 4.84–4.92 (m, 1H), 6.17 (s, 2H), 7.46 (s, 1H), 7.66 (s, 1H), 7.77 (s, 1H), 7.87 (s, 1H), 9.35 (s, 1H); (**3c**) 1.80–2.21 (m, 4H), 3.63–3.82 (m, 2H), 4.07 (s, 3H), 4.14 (s, 3H), 4.52–4.84 (m, 3H), 6.18 (s, 2H), 7.48 (s, 1H), 7.70 (s, 1H), 7.90 (s, 1H), 8.04 (s, 1H), 9.39 (s, 1H); (**4a**) 2.42(s, 6H), 3.04 (t, 2H, $J=7.2$ Hz), 4.08 (s, 3H), 4.17 (s, 3H), 4.64 (t, 2H, $J=7.2$ Hz), 6.25 (s, 2H), 7.81 (s, 1H), 7.84 (s, 1H), 8.07 (s, 1H), 8.65 (s, 1H); (**4b**) 1.96–1.98 (m, 9H), 2.79 (dd, 1H, $J=12.2$, 7.2 Hz), 3.26 (dd, 1H, $J=12.0$, 7.8 Hz), 4.08 (s, 3H), 4.18 (s, 3H), 4.80–4.88 (m, 1H), 6.22 (s, 2H), 7.66 (s, 1H), 7.80 (s, 1H), 7.82 (s, 1H), 8.58 (s, 1H); (**4c**) 1.74–2.33 (m, 4H), 3.74–4.0 (m, 2H), 4.09 (s, 3H), 4.18 (s, 3H), 4.56–4.70 (m, 3H), 6.25 (s, 2H), 7.80 (s, 1H), 7.84 (s, 1H), 8.32 (s, 1H), 8.63 (s, 1H); (**4d**) 1.07 (t, $J=7.4$ Hz, 3H), 1.56 (m, 2H), 2.14 (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).
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