Nitro and Amino Substitution in the D-Ring of 5-(2-Dimethylaminoethyl)-2,3-methylenedioxy-5*H*-dibenzo[*c,h*][1,6]naphthyridin-6-ones: Effect on Topoisomerase-I Targeting Activity and Cytotoxicity

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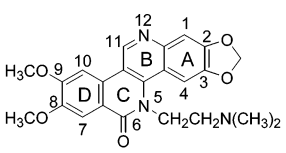
5*H*-8,9-Dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c,h*][1,6]naphthyridin-6-one exhibits potent TOP1-targeting activity and pronounced antitumor activity. It was hypothesized that replacement of the two methoxyl groups with a nitro substituent would allow for retention of similar activity. In this study 8-, 9-, and 10-nitro-5*H*-2,3-methylenedioxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c,h*][1,6]naphthyridin-6-one and their amino derivatives were synthesized and assessed for their relative TOP1-targeting activity and cytotoxicity. In the case of both the 8- and 9-nitro analogues, their TOP1-targeting activity and cytotoxicity are greater than that of camptothecin and comparable to that of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c,h*][1,6]naphthyridin-6-one.

Introduction

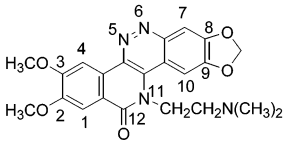
Topoisomerases are nuclear enzymes critical to replication and transcription. There are two major subtypes, topoisomerase I (TOP1) and topoisomerase II (TOP2) based upon differences in their initial mechanisms wherein a single- or double-stranded DNA break is implicated.[1−3] Topoisomerase-targeting agents that stabilize the cleavable complex formed between the enzyme and DNA have proved to be effective in the treatment of cancer. Small molecules capable of stabilizing the enzyme−DNA cleavable complex convert these nuclear enzymes into cellular poisons.

Camptothecin was the first agent identified as a TOP1-targeting agent.[4] There are two clinical agents, topotecan (Hycamtin) and irinotecan (CPT-11/Camptosar), that have been developed from the research performed with camptothecin and its structurally related analogues. These camptothecin-based drugs have a γ-lactone incorporated within their structures, which is susceptible to hydrolysis. Ring-opening of the camptothecin lactone moiety results in the formation of 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.[8−11]

Several novel non-camptothecin TOP1-targeting agents have been identified in recent years. These include derivatives of bi- and terbenzimidazoles,[12,13] benz[*a*]-anthracenes,[14] benzo[*c*]phenanthridine and protoberberine alkaloids,[15,16] indolocarbazoles,[17] the fungal metabolites bulgarein[18] and saintopin,[19] and several indenoisoquinolines[20] and benzophenazines.[21] Studies in our laboratory have demonstrated that 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c,h*][1,6]naphthyridin-6-one, **1**, and 11*H*-2,3-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-8,9-methylenedioxy-5,6,11-triazachrysen-12-one, **2**, can exhibit potent TOP1-targeting activity and pronounced cytotoxicity.[22,23] Potent antitumor activity was observed for **1** administered orally and parenterally to athymic nude mice bearing the human tumor xenograft, MDA-MB-435.



1



2

Structure−activity studies on variously substituted benzo[*i*]phenanthridines and dibenzo[*c,h*]cinnolines have demonstrated that the presence of a nitrogen heteroatom adjacent to a benzo-ring that possesses a methylenedioxy group is important for maintaining potent TOP1-targeting activity.[24−26] In addition, substitution of two vicinal methoxyl substituents within the A-ring of benzo[*i*]phenanthridine derivatives, specifically at the 2,3-positions, has also proven to be a structural feature associated with potent TOP1-targeting activity.[25] It was speculated that replacement of the vicinal methoxyl groups with a nitro substituent could potentially allow for retention of similar biological activity. The synthetic methods previously developed in our laboratory for the preparation of various benzo[*i*]phenanthridine and dibenzo[*c,h*]cinnoline derivatives, however, are incompatible

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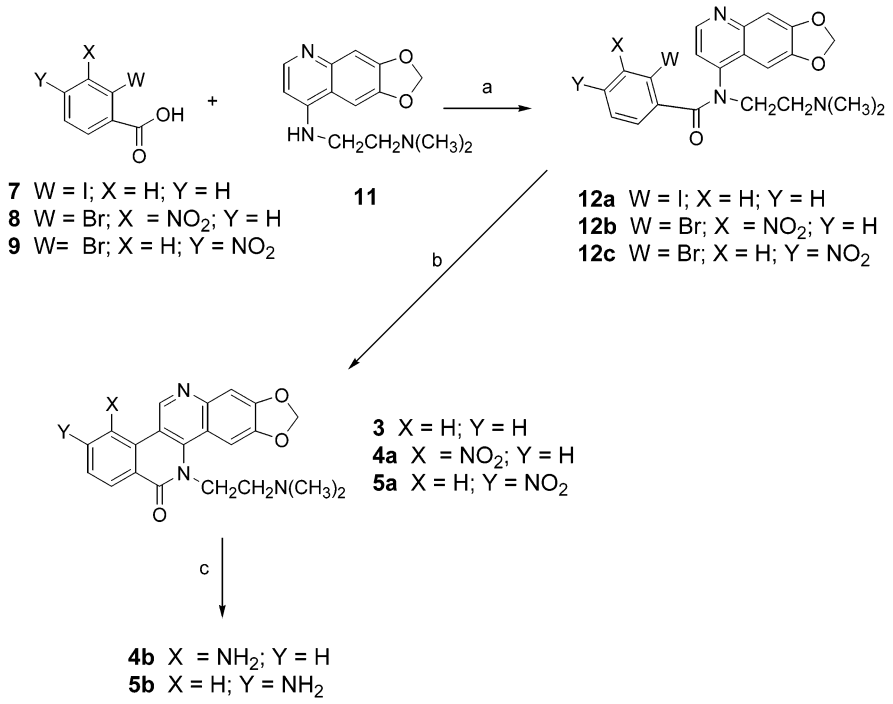
Scheme 1[a]



[a] (a) $(COCl)_2$, CH_2Cl_2, **11**, and then TEA, CH_2Cl_2; (b) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, DMF; (c) RaNi, $H_2NNH_2 \cdot H_2O$.

with the formation of such nitro analogues.[24−26] The synthetic approach employed herein for the preparation of 5-substituted 5*H*-8,9-dimethoxy-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-ones permits the introduction of a nitro moiety at or adjacent to the sites occupied by the methoxyl substituents. The present study was undertaken to determine if one or more such nitro derivatives could retain similar TOP1-targeting activity as **1**. In this study we synthesized 8-, 9-, and 10-nitro-5*H*-2,3-methylenedioxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo[*c*,*h*][1,6]naphthyridin-6-one as well as their amino derivatives to assess their relative TOP1-targeting activity and cytotoxicity.

Chemistry

The synthetic methods for the preparation of 5*H*-2,3-methylenedioxy-5-(2-*N*,*N*-dimethylaminoethyl)dibenzo-[*c*,*h*][1,6]naphthyridin-6-one, **3**, and its 8-, 9-, and 10-nitro derivatives, **4a**, **5a**, and **6a** are outlined in Schemes 1 and 2. The *o*-bromobenzoic acids **7**−**10** were available commercially or prepared as reported in the literature.[27] Reaction of 4-chloroquinoline with *N*,*N*-dimethylethylenediamine in the presence of phenol provided the 4-alkylaminoquinoline, **11**. As outlined in Schemes 1 and 2, the *o*-bromobenzoic acids, **7**−**9**, were converted to their acid chlorides and reacted with **11** to form the requisite *o*-iodobenzamide intermediates, **12a**−**d**. Treatment of **12a**−**c** under Heck cyclization conditions[28] resulted in the formation of **3**, **4a**, or **5a** as the major product in yields ranging from 34 to 38%. Reduction of **4a** and **5a** with Ra−Ni in the presence of hydrazine provided the amino derivatives, **4b** and **5b**.

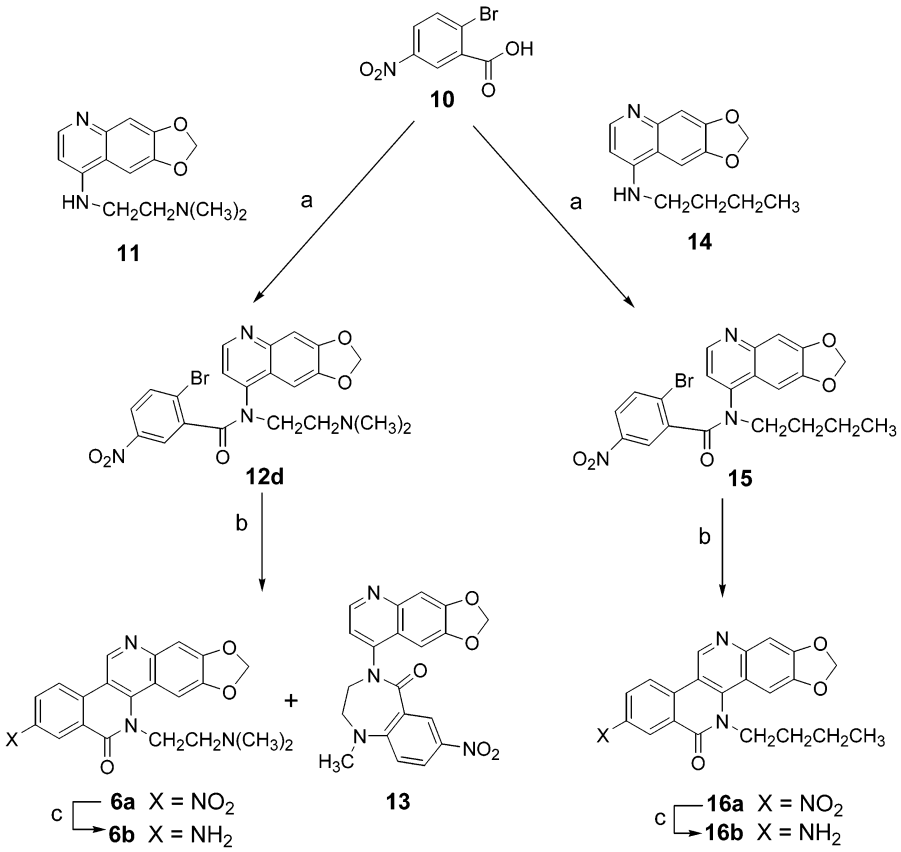
Reaction of **12d** under identical cyclization conditions, as shown in Scheme 2, resulted in the formation of **6a** together with **13** as a major side product. The involvement of the tertiary amine in the formation of this side-product was demonstrated using the *N*-butyl-*o*-bromobenzamide, **15**, which under Heck cyclization conditions only formed the desired methylenedioxydibenzo[*c*,*h*][1,6]-naphthyridin-6-one, **16a**. This byproduct is most likely formed by intramolecular nucleophilic substitution by the tertiary alkylamine on the electrophilic *p*-nitroaryl bromide, which is sterically unhindered in the case of **12d**, followed by elimination of methyl bromide. In the case of both **6a** and **16a**, treatment with Ra−Ni and hydrazine provides the corresponding amino analogues **6b** and **16b**.

Biological Results and Discussion

Results from the evaluation of the relative TOP1-targeting activity and cytotoxicity of **3** and its nitro derivatives, **4a**−**6a**, and amino derivatives, **4b**−**6b**, are provided in Table 1. In comparing their intrinsic TOP1-targeting activity relative to the 8,9-dimethoxy analogue **1**, it is evident that both **5a** and **6a** are more potent. Both **5a** and **6a** are more potent than camptothecin as TOP1-targeting agents. In contrast, **3** is at least 1 order of magnitude less active than **1** as a TOP1-targeting agent and the 10-nitro derivative **4a** does not exhibit any significant TOP1-targeting activity. The side-product **13**, formed in the synthesis of **6a**, does not possess significant TOP1-targeting activity.

Compounds **5a** and **6a** have comparable cytotoxicity to that of **1** toward the human lymphoblast tumor cell line, RPMI8402. Both **5a** and **6a** possess greater cytotoxicity than either camptothecin or topotecan in this cell line. They also exhibit similar cross-resistance to the camptothecin-resistant variant of RPMI8402, CPT-K5. Consistent with its lower potency as a TOP1-targeting agent, **3** is significantly less cytotoxic toward RPMI8402 than **1**. Compound **4a** is more than 3 orders of magnitude less cytotoxic toward RPMI8402 than either **1**, **5a**, or **6a**, consistent with its lack of significant TOP1-targeting activity. The extent of DNA fragmenta-

Scheme 2[a]



(a) $(COCl)_2$, CH_2Cl_2, **11**, and then TEA, CH_2Cl_2; (b) $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Ag_2CO_3, DMF; (c) RaNi, $H_2NNH_2 \cdot H_2O$.

Table 1. TOP1-Targeting Activity and Cytotoxicity

compound	TOP1-mediated DNA cleavage (REC)[a]	cytotoxicity (IC_{50}; μM)[b] RPMI8402	CPT-K5
1	0.5	0.002	0.9
2	0.3	0.001	0.6
3	6	0.06	0.95
4a	1000	4.8	> 10
4b	15	0.34	3.2
5a	0.1	0.002	2.8
5b	0.5	0.025	1.0
6a	0.2	0.003	0.33
6b	8.0	0.08	0.38
13	1000	3.0	> 10
16a	10	0.24	> 10
16b	500	1.25	> 10
Camptothecin	0.5	0.005	61
CPT-11	25	0.57	>100
Topotecan	1	0.012	> 50
VM-26	>1000	0.22	0.28

[a] TOP1 cleavage values are reported as REC, relative effective concentration, i.e., concentrations relative to topotecan, whose value is arbitrarily assumed as 1, that produce the same degree of cleavage of the plasmid DNA in the presence of human TOP1. [b] IC_{50} was calculated after 4 days of continuous drug exposure and are the average of two or more replicate assays.

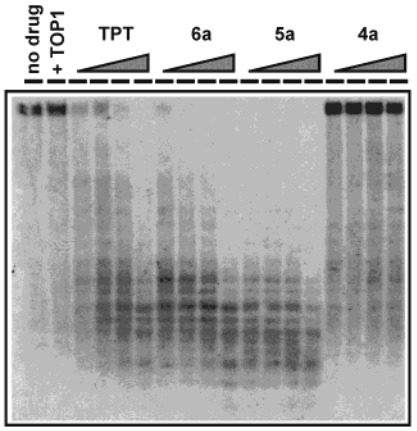


Figure 1. Stimulation of enzyme-mediated DNA cleavage by topotecan (TPT), **6a**, **5a**, **4a** using human TOP1. The first lane is DNA control without enzyme. The second lane is the control with enzyme alone. The rest of the lanes contain human TOP1 and serially (10-fold each) diluted compound from 0.001 to 1.0 μM.

tion that occurs in the presence of TOP1 and various concentrations of **4a**, **5a**, and **6a** can be seen in Figure 1.

The TOP1-targeting activities of the amino analogues **5b** and **6b** are 5- and 40-fold less, respectively, than their nitro derivatives **5a** and **6a**. This difference is reflected in their decreased cytotoxic activity toward RPMI8402 relative to their nitro analogues. In the case of **4b**, however, there was a significant enhancement in TOP1-targeting activity and cytotoxicity relative to its nitro analogue, **4a**. The steric interactions between the nitro group that resides within the bay-region (position 10−10a−10b−11) and the hydrogen atom attached at C11 may force the nitro group out of the plane of the benzo-ring. This alteration in topology may not be favored for retention of TOP1-targeting activity. Reduction of the nitro group reduces the steric interaction that can occur within this bay-region and could be responsible for the enhancement observed for **4b**

relative to **4a** in both TOP1-targeting activity and cytotoxicity.

Conclusions

These data indicate that a nitro substituent at either the 8- or the 9-position can replace the vicinal methoxyl groups of 5*H*-8,9-dimethoxy-5-(2-*N*,*N*-dimethylaminoethyl)-2,3-methylenedioxydibenzo[*c*,*h*][1,6]naphthyridin-6-one with retention of its potent TOP1-targeting activity and cytotoxicity. In light of the number of non-camptothecin TOP1-targeting agents that possess vicinal methoxyl groups such as coralyne, nitidine, benzo[*i*]-phenanthridines, benzo[*c*,*h*]cinnolines, and indenoisoquinolines, this modification could potentially allow for the development of several new highly active analogues with significantly altered electronic properties. The loss of activity associated with the positional isomer **4a** provides some insight into both the steric tolerance and molecular topology that is associated with retention of TOP1-targeting activity. These structure−activity data should assist further studies focused on establishing an understanding of the structural alignment and molecular interactions associated with the stabilization of the ternary complex between DNA, TOP1, and these novel TOP1-targeting agents.

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Supporting Information Available: Synthetic procedures, spectral and analytical data for all new compounds, and experimental details of in vitro assays. This material is available free of charge via the Internet at http://pubs.acs.org.

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