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Synthesis of nitrated indenoisoquinolines as topoisomerase I inhibitors

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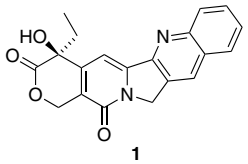
Abstract—Indenoisoquinolines and dihydroindenoisoquinolines have been synthesized possessing a nitro-substituted isoquinoline ring in an effort to explore the effects of electron-withdrawing substituents on biological activity. The in vitro anticancer activities of these molecules have been tested in the National Cancer Institute's screen of 55 cell lines. The compounds have also been tested for topoisomerase I (top1) inhibition. The results indicate that these substances are a potent class of top1 inhibitors with sub-micromolar cytotoxicity mean graph midpoints (MGM) and top1 inhibition equal to camptothecin.

The indenoisoquinolines are a class of cytotoxic molecules that have been demonstrated to inhibit topoisomerase I (top1) by intercalating between DNA bases at the enzyme's cleavage site.[1] This mechanism of action is identical to the natural product camptothecin (**1**) and its clinically useful derivative topotecan (**2**).[1] Thus, the indenoisoquinolines constitute a novel class of non-camptothecin top1 inhibitors. Mechanistically, the intercalation of these molecules elongates the DNA such that top1 cannot catalyze the religation of the DNA backbone.[2] These inhibitors are therefore classified as top1 'poisons' as opposed to top1 'suppressors', which inhibit the DNA cleavage reaction.



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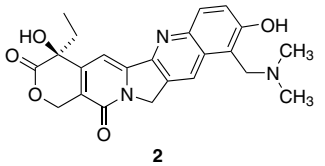

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Historically, our laboratory has synthesized indenoisoquinolines with methylenedioxy and di(methoxy) substituents as well as molecules that lack substituents on both the isoquinoline and indenone rings.[1,3–7] Past synthetic efforts have focused on exploring the substitution pattern of the lactam nitrogen with a wide variety of carbon and heteroatom substituents.[3–5,7] These efforts have rewarded our laboratory with the ability to potentiate the cytotoxicity and top1 inhibition of the indenoisoquinolines through the prudent selection of functionalities protruding from the lactam nitrogen.

Molecular modeling of the indenoisoquinolines in a ternary complex with DNA and top1 indicates few hydrogen-bonding contacts between the polycyclic backbone of the inhibitor and top1.[7] Thus, if one keeps strictly with the indenoisoquinoline pharmacophore, there is little chance of increasing the potency of these compounds using hydrogen-bonding contacts alone. Examination of the recently published crystal structure comprising topotecan, DNA, and top1 suggests that approximately 60% of the solvent-accessible surface of topotecan is involved in π-stacking interaction with the DNA bases above and below insertion.[2] Therefore, it is reasonable to conclude that the intercalation of topotecan, and by analogy the indenoisoquinolines, is driven

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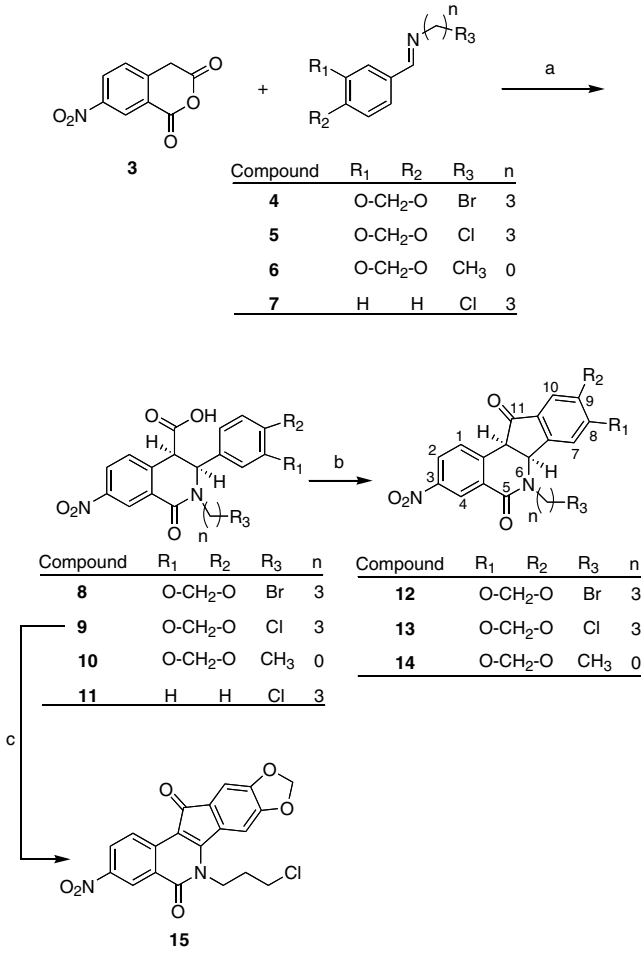
in large part by favorable π-stacking interactions between the DNA bases and the aromatic rings of the molecule.[8] If one could effectively increase a molecule's affinity for π-stacking, then it would constitute a viable method for increasing the potency of topotecan, and by analogy, the indenoisoquinolines. Literature precedence abounds for the interaction of nitrated aromatic compounds with DNA, especially with regard to the intercalation and mutagenicity of these molecules.[9] However, the purposeful attempt to capitalize on increased π-stacking interactions with respect to top1 inhibitors has not been described.

At this time, we would like to report our renewed interest in the polycyclic backbone of the indenoisoquinolines, especially with respect to the substitution of the isoquinoline and indenone aromatic rings. Our laboratory's precedented choice of aromatic functionalization, the methylendioxy and di(methoxy) substituents, have all been capable of donating electron density into the aromatic rings of the molecule. The present investigation was undertaken in order to determine if the introduction of an electron-withdrawing nitro substituent on the isoquinoline ring of the indenoisoquinolines would increase the molecule's biological activity by capitalizing on potential π-stacking interactions.

Carboxylic acids **8**, **9**, **10**, and **11** were efficiently prepared through condensation of the appropriate Schiff bases with 4-nitrohomophthalic anhydride[10] (**3**) (Scheme 1). Treatment of these carboxylic acids with our group's protocol[11] of thionyl chloride induced Friedel–Crafts ring closure and dehydrogenation, however, resulted in a nonexistent or poor yield of the corresponding indenoisoquinolines. Alternative methods (Eaton's reagent[3] and polyphosphoric acid[12]) were subsequently sought to affect 'Friedel–Crafts' type ring closure to provide dihydroindenoisoquinolines **12**, **13**, and **14**. However, ring closure was finally accomplished using P_2O_5 as a dehydrating agent[11] to provide the corresponding dihydroindenoisoquinolines.



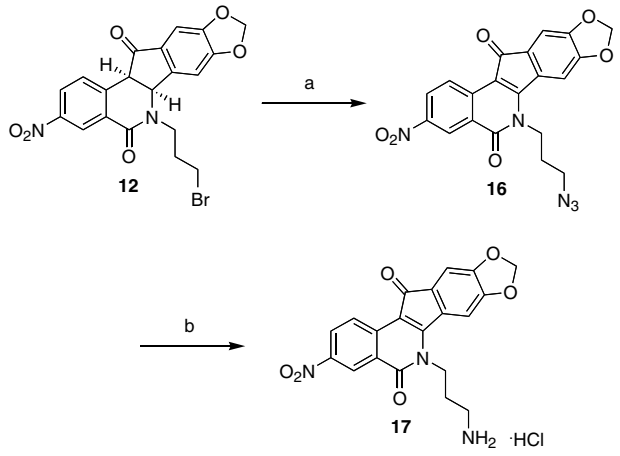
Compound	R_1	R_2	R_3	n
4	O-CH_2-O		Br	3
5	O-CH_2-O		Cl	3
6	O-CH_2-O		CH_3	0
7	H	H	Cl	3

Compound	R_1	R_2	R_3	n
8	O-CH_2-O		Br	3
9	O-CH_2-O		Cl	3
10	O-CH_2-O		CH_3	0
11	H	H	Cl	3

Compound	R_1	R_2	R_3	n
12	O-CH_2-O		Br	3
13	O-CH_2-O		Cl	3
14	O-CH_2-O		CH_3	0

Scheme 1. Reagents and conditions: (a) $CHCl_3$, 57–84%; (b) P_2O_5, $CHCl_3$, reflux, 28–50%; (c) $SOCl_2$, 17%.

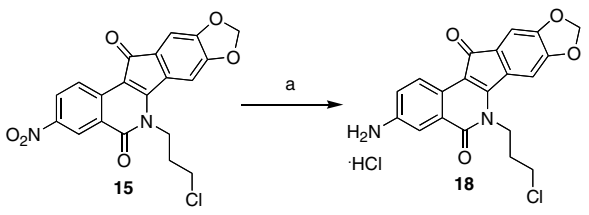
In an effort to introduce a propylamine substituent on the lactam nitrogen, dihydroindenoisoquinoline **12** was treated with NaN_3 in DMSO. This procedure provided the corresponding alkyl azide substituent, but unexpectedly oxidized **12** from the dihydroindenoisoquinoline skeleton to the indenoisoquinoline skeleton (Scheme 2). Investigations into this unexpected oxidation are currently underway. Staudinger reduction[4] of azide **16** proceeded smoothly to provide **17**, which was isolated as its hydrochloride salt.



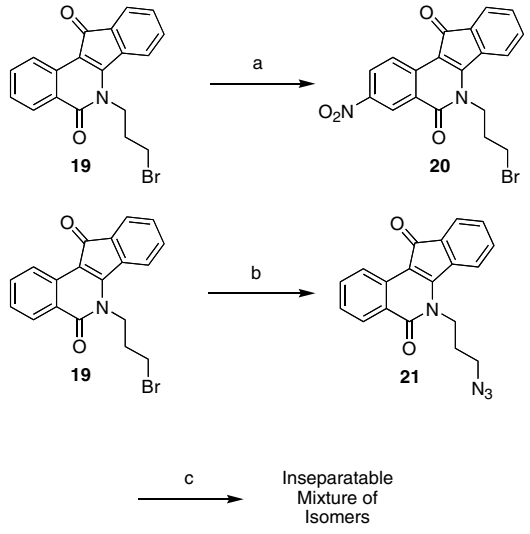
Scheme 2. Reagents and conditions: (a) NaN_3, DMSO, 80%; (b) (1) $P(OEt)_3$, benzene, reflux; (2) 3 N HCl/MeOH, reflux, 74%.

As a negative control for the electron-withdrawing effects of the nitro-substituted isoquinoline ring, compound **18** was synthesized (Scheme 3) with the nitro substituent replaced with an electron-donating aniline-type amino functionality.

Nitrated indenoisoquinolines lacking the methylenedioxy ring were initially synthesized as described in Scheme 4. However, due to the low yield and difficulty in separating isomers, synthetic efforts shifted toward developing a new method to create the indenone ring. Compound **11** seemed to be a suitable intermediate for this venture, provided that an intramolecular Friedel–Crafts acylation could be accomplished. It was envisioned that reaction conditions could be found that would provide the dihydroindenoisoquinoline skeleton, which could then be oxidized to the indenoisoquinoline

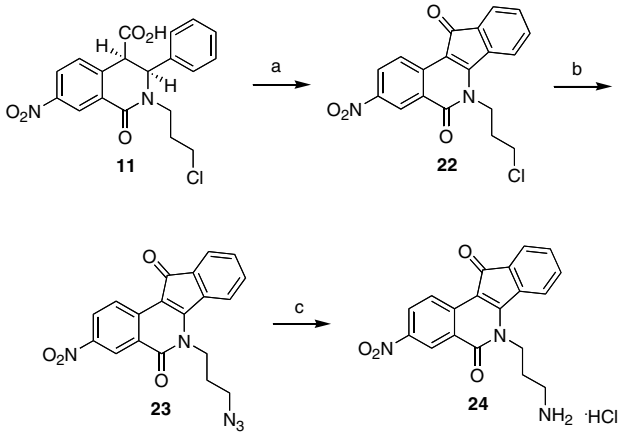


Scheme 3. Reagents and conditions: (a) (1) Raney Ni, HO_2CH, acetone, DMF; (2) 2 M HCl/ether; 40%.



Scheme 4. Reagents and conditions: (a) Nitrated silica, $CHCl_3$, reflux, 17%; (b) NaN_3, DMSO, 87%; (c) nitrated silica, $CHCl_3$, reflux.

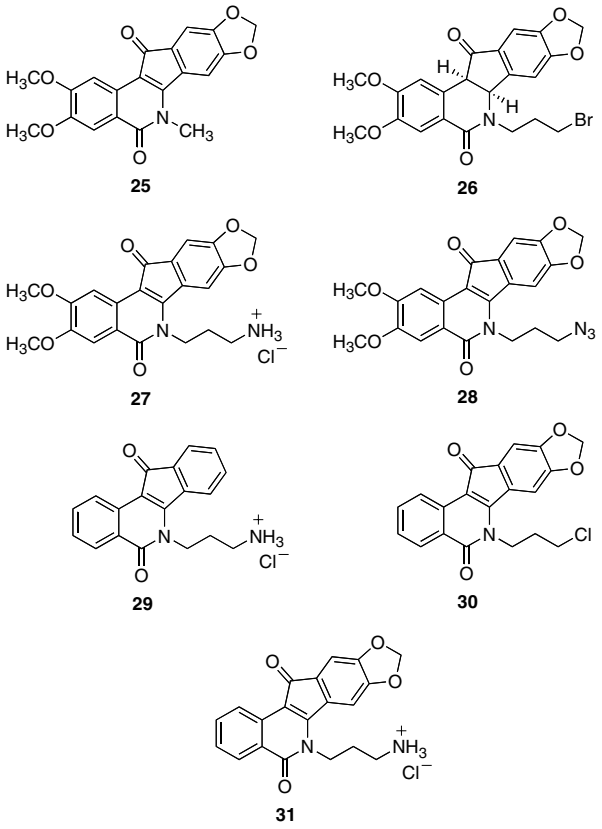
skeleton in a subsequent chemical transformation. Ultimately, compound **22** was unexpectedly synthesized (Scheme 5) after initial treatment with thionyl chloride, followed by aluminum chloride. Interestingly, this protocol marks the second[11] oxidative Friedel–Crafts acylation method discovered to date in our laboratory and it is currently being extended to the synthesis of other indenoisoquinolines while defining its scope and synthetic utility. The exact sequence of steps involving: (1) acid



Scheme 5. Reagents and conditions: (a) (1) $SOCl_3$, benzene; (2) $AlCl_3$, 57%; (b) NaN_3, DMSO, 100 pC; 98%; (c) (1) $P(OEt)_3$, benzene, reflux; (2) 3 N HCl/MeOH, reflux, 86%.

chloride formation, (2) dehydrogenation, and (3) intramolecular Friedel–Crafts acylation is currently under investigation.

The indenoisoquinolines were examined for antiproliferative activity against the human cancer cell lines in the National Cancer Institute screen, in which the activity of each compound was evaluated with approximately 55 different cancer cell lines of diverse tumor origins. The GI50 values obtained with selected cell lines, along with the mean graph midpoint (MGM) values, are summarized in Table 1. The MGM is based on a calculation of the average GI50 for all of the cell lines tested (approximately 55) in which GI50 values below and above the test range (10^{-8} to 10^{-4} M) are taken as the minimum (10^{-8} M) and maximum (10^{-4} M) drug concentrations used in the screening test. For comparison purposes, the activity of the previously reported lead compound **25**[1] is included in the table. The relative potencies of the compounds in the production of topoisomerase I-mediated DNA cleavage are also listed in the table. Several of our previously synthesized compounds are included in Table 1 for the sake of comparison with the presently reported top1 inhibitors. These include the indenoisoquinolines **26**,[5] **27**,[4] **28**,[4] **29**,[14] **30**,[4] and **31**.[14]



The data in Table 1 suggests that the incorporation of a nitro substituent at the 3-position of the isoquinoline ring has a profoundly enhancing effect on cytotoxicity and top1 inhibition. The activities of dihydroindenoisoquinolines **12** and **13** (16 and 18 nM, respectively), in

Table 1. Cytotoxicities and topoisomerase I inhibitory activities of indenoisoquinoline analogues[13]

Compound	Cytotoxicity (GI50 in μM)[a]								MGM[b]	Top1 cleavage[c]
	Lung HOP-62	Colon HCT-116	CNS SF-268	Melanoma UACC-62	Ovarian OVCAR-3	Renal SN12C	Prostate DU-145	Breast MDA-MB-435		
12	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.016 ± 0.003	+
13	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.018 ± 0.001	+
14	0.158	0.014	0.024	0.026	0.03	0.033	0.05	0.107	0.105 ± 0.007	+++
15	0.41	2.4	0.148	0.229	9.12	0.132	0.098	1.82	0.832	+++
16	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.098 ± 0.06	++++
17	<0.010	<0.010	<0.010	0.017	0.302	<0.010	<0.010	0.025	0.090 ± 0.04	++++
18	NT	NT	>100	>100	>100	>100	NT	>100	25	0
19	4.59	4.47	4.61	10.1	23.5	7	13	21.5	13.3 ± 4.80	±
20	NT	3.45	>100	>100	>100	>100	>100	>100	40	0
21	7.59	4.9	NT	19.5	7.94	25.1	44.7	7.76	12.3	NT
22	43.7	3.63	23.4	20.4	4.9	26.9	40.7	31.6	18.2	NT
23	>100	8.13	79.4	44.7	NT	72.4	>100	>100	13.5	NT
24	0.275	0.085	0.302	0.372	0.112	0.102	0.148	0.832	0.245	NT
25	1.3	35	NT	4.2	73	68	37	96	20	++
26	0.12	0.07	NT	0.05	0.4	0.03	0.03	0.15	0.12 ± 0.03	+
27	0.06	0.13	NT	0.25	0.31	0.31	0.04	1.21	0.16	+++
28	3.58	7.4	NT	3.13	76.2	4.53	3.19	>100	11.8 ± 0.82	++
29	0.2	0.18	NT	0.26	1.38	0.16	0.22	0.78	0.32 ± 0.23	+++
30	13.1	74	NT	53.8	66.3	>100	12.7	>100	35.1 ± 2.00	NT
31	0.28	0.68	NT	0.18	1.45	0.19	0.06	1.82	0.25 ± 0.05	+

The activity of the compounds to produce top1-mediated DNA cleavage was expressed semi-quantitatively as follows: +: weak activity; ++: similar activity as the parent compound **25**; +++ and ++++: greater activity than the parent compound **25**; ++++: similar activity as 1 μM camptothecin.
[a] The cytotoxicity GI50 values are the concentrations corresponding to 50% growth inhibition.
[b] Mean graph midpoint for growth inhibition of all human cancer cell lines successfully tested.
[c] The compounds were tested at concentrations ranging up to 10 μM.

particular, are each an order of magnitude greater than their di(methoxy) substituted counterpart **26**. To date, compounds **12** and **13** are the most cytotoxic molecules synthesized by our laboratory. Predictably, the dihydroindenoisoquinolines were less active than their indenoisoquinoline counterparts in the top1 inhibition assay, most likely due to the strict requirement of planarity for potent top1 inhibition. This trade-off between cytotoxicity in the cellular assay and inhibition of top1 in the cell-free assay (compare **13** and **15**) has been observed by our group in the past.[3,5] A possible explanation for the difference may be that the dihydroindenoisoquinolines, especially those with nitro substituents, could be serving as prodrugs in the cell-based cytotoxicity assay where differences in uptake and distribution could cause the enhanced cytotoxicity. After distribution within the cell, these dihydro prodrugs could be oxidized to the indenoisoquinolines, which could then inhibit top1. Although further investigation into this possible explanation is ongoing, the readily occurring oxidation for the transformation of **12** to **16** (Scheme 2) could be an allusion to an oxidative process occurring within the cell.

With regard to indenoisoquinolines such as **15**, **16**, and **17** that posses a nitro substituent on the isoquinoline ring and a methylenedioxy-substituted indenone ring, these molecules display superb cytotoxicities (0.832 μM, 98, and 90 nM, respectively) and top1 inhibition (+++, ++++, and ++++, respectively). These compounds outperformed the dihydroindenoisoquinolines in the top1 inhibition assay and were superior in all respects to parent compound **25** and analogues **27**, **28**, **30**, and **31** that lack the nitro substituent. Compound **16**, in particular, deserves special recognition since it marks the first azido-substituted indenoisoquinoline to possess sub-micromolar cytotoxicity and top1 inhibition similar to camptothecin. In an effort to further define the effect of the nitro substituent, compound **18** (25 μM MGM) was prepared with the nitro functionality replaced with an aniline-type nitrogen. As indicated in Table 1, activity across all cell lines was lost upon switching from an electron-withdrawing to an electron-donating substituent. In fact, the presence of the aniline functionality was hardly better than removing the substituent entirely as in compound **30** (25 vs 35 μM MGM).

Removal of the methylenedioxy substituent from the indenone ring proved to be detrimental for the analogues presently disclosed. A direct comparison of compounds **16** (98 nM) and **23** (13.5 μM) indicates that a synergistic effect is occurring between the nitro and methylenedioxy substituents. However, removal of both the nitro and methylenedioxy groups (represented by compounds **19**, **21**, and **29**) proved to be detrimental to activity until a propylamine lactam substituent could be introduced (**29**). The enhancing effect provided by alkylamine substituents at the lactam position has previously been disclosed by our group.[7] Interestingly, nitrated compounds **20**, **22**, and **23** (40, 18.2, and 13.5 μM, respectively) displayed the same ill-fated trend, only to regain activity when alkylamine **24** (0.245 μM) was prepared. This data further supports the past structure–activity relationships (SAR) determined in our labora-

tory and alludes to an important role for the methylenedioxy ring, especially when the isoquinolone moiety is nitrated.

In summary, a new class of indenoisoquinolines and dihydroindenoisoquinolines has been synthesized that possess a nitro substituent on the isoquinolone ring. The molecules have been tested for cytotoxicity in the NCI's 55 cell line screen and for top1 inhibition. Results indicate that these substances are the most potent class of indenoisoquinolines synthesized to date and ongoing investigations are being performed to increase understanding and exploit the substitution of indenoisoquinolines and dihydroindenoisoquinolines with electron-withdrawing substituents. Future emphasis will be focused towards supporting the electron-withdrawing/π-stacking hypothesis for increasing the affinity of the indenoisoquinolines in the DNA cleavage site of the ternary complex.

Acknowledgements

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