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Synthesis of benz[*d*]indeno[1,2-*b*]pyran-5,11-diones: Versatile intermediates for the design and synthesis of topoisomerase I inhibitors

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Abstract—A method has been developed that relies on a two-step, one-pot condensation between phthalide and 2-carboxybenzaldehydes to provide benz[*d*]indeno[1,2-*b*]pyran-5,11-diones in a multi-gram fashion. Treatment of these compounds with a primary amine allows rapid access to various N-substituted indenoisoquinolines, whose in vitro anticancer activity and topoisomerase I inhibition have been evaluated.

The topoisomerase I (Top1) inhibitory activity of indenoisoquinolines was discovered after a COMPARE analysis of the cytotoxicity profile of an indenoisoquinoline lead compound revealed a strong correlation to those of other known Top1 inhibitors, including camptothecin (**1**) and its clinically useful derivatives topotecan (**2**) and irinotecan (**3**).[1] Additionally, the simplified indenoisoquinoline oracin (**4**) has garnered interest as an anticancer therapeutic for its ability to induce G2 cell cycle arrest and apoptosis in Burkitt's lymphoma cells (Fig. 1).[2–7]

The indenoisoquinolines, like the camptothecins, stabilize DNA–Top1 cleavage complexes by intercalating at the DNA cleavage site, resulting in inhibition of the re-ligation reaction.[1,8,9] This classifies the indenoisoquinolines as Top1 'poisons' as opposed to Top1 'suppressors', which inhibit Top1's ability to cleave the phosphodiester backbone of DNA. There are obvious structural differences between the indenoisoquinolines and the camptothecins, and these differences give rise to several elements that warrant the further development of indenoisoquinolines as Top1 anticancer therapeutics. Namely, the two

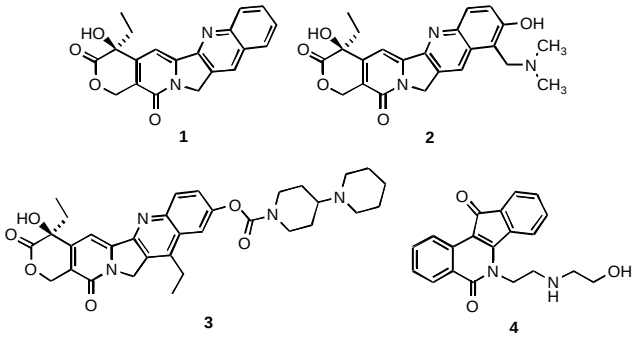

Figure 1. Representative topoisomerase I inhibitors.

molecular classes display alternative cleavage site specificities that could result in a different antitumor spectrum.[1] Second, the cleavage complexes induced by indenoisoquinolines are more stable than those formed by the camptothecin family.[1,8] Lastly, the camptothecins are chemically unstable due to hydrolysis of their lactone ring, with the resulting hydroxy-acid product displaying a high affinity for serum albumin.[10]

Previous work has been concerned with the synthesis of unsubstituted indenoisoquinolines utilizing a condensation between benz[*d*]indeno[1,2-*b*]pyran-5,11-dione (**8**) (referred to below as an indenopyran) and a primary

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amine.[11] Ultimately, the stockpile of indenopyran **8** has begun to dwindle and faced with the fact that indenopyran **8** is no longer commercially available, potential syntheses of this material were initiated. Fortunately, this molecule has been of considerable synthetic interest since the 1960s with the first 'direct' synthesis performed by Pailer.[12] Several 'indirect' syntheses were performed by Wawzonek[13–17] in pursuit of a dibenzo[*a*,*e*]cylcooctenetrione and this synthesis was subsequently confirmed by Yates[18,19] to provide the unsubstituted indenopyran instead of the desired trione. Although the initial effort was misguided, Wawzonek was the first to report the synthesis of an indenoisoquinoline from an indenopyran and to document the cytotoxicity of the product.[17]

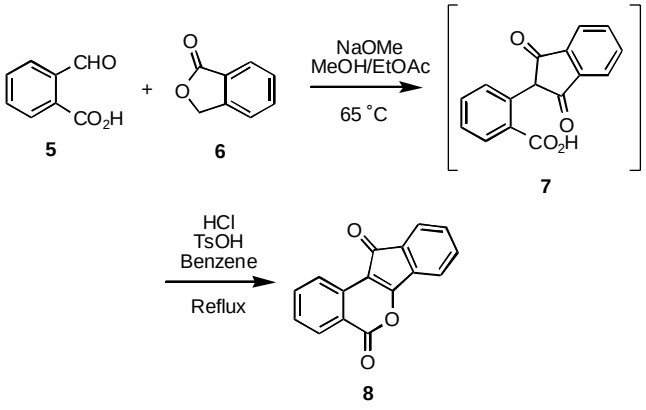
Pailer's synthesis represented the most logical choice for obtaining the desired indenopyran **8** (three steps, 31% yield).[12] Since this initial synthesis, however, an improved method of obtaining intermediate **7** (Scheme 1) was reported by Shapiro and colleagues in their syntheses of indandione anticoagulants.[20,21] Thus, a synthetic plan was devised to incorporate both methods and was intended to provide an improved synthesis of indenopyran **8**. Not only did this occur, but during the course of this research a protocol was devised that was capable of accomplishing the synthesis as a one-pot two-step method with an improved yield (86%) compared to the previously reported synthesis[12] (Scheme 1).

Condensation of phthalide (**6**) with phthaldehydic acid **5** using Shapiro's method[21] provided compound **7**, which upon acidic treatment could be efficiently converted to lactone **8** in a single pot with high yield. Following the traditional method, intermediate **7** could be isolated and subsequently cyclized to provide indenopyran **8** according to the conditions illustrated in Scheme 1, but this provided no synthetic advantage.

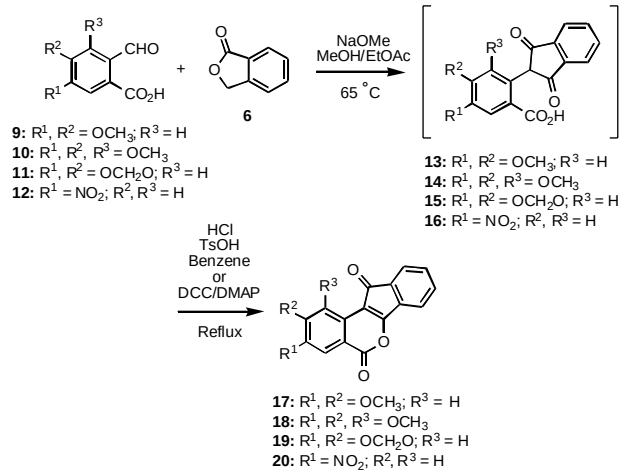
Pleased with this new development, curiosity led to the extrapolation of this new method to incorporate substituted phthaldehydic acids for the production of new indenopyrans. As a preliminary investigation, phthaldehydic acids **9**–**12** were chosen for incorporation based on the use of these functionalities in previous research for reasons already disclosed.[11,22] Although the synthetic method provided substituted indenopyrans **17**–**20**, the yields were diminished with respect to the unsubstituted variant **8** (86%, 18%, 7%, 78%, and 31% yields for compounds **8**, **17**, **18**, **19**, and **20**, respectively). However, no optimization was performed in this work, with initial efforts providing sufficient quantities of **17**–**20** for biological testing and **17**, **19**, and **20** for additional analogue syntheses (Scheme 2).

With the synthesis of substituted indenopyrans accomplished, the focus of the research shifted to the use of these compounds as advanced intermediates for the rapid and efficient generation of indenoisoquinolines. In almost all cases, treatment of the requisite indenopyran with a suitable primary amine resulted in the formation of the corresponding indenoisoquinoline in high yield (Scheme 3).[11]

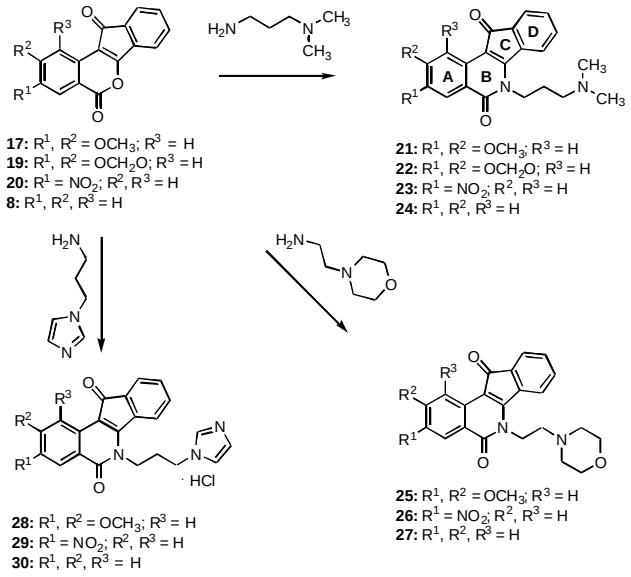
The indenopyrans and indenoisoquinolines were examined for antiproliferative activity against the



Scheme 1. One-pot synthesis of indenopyran **8**.



Scheme 2. Synthesis of substituted indenopyrans.



Scheme 3. Synthesis of indenoisoquinolines.

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

Compound	Cytotoxicity (GI_{50} in μM)[a]								MGM[b]	Top 1 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		
1	0.01	0.03	0.01	0.01	0.22	0.02	0.01	0.04	0.0405 ± 0.0187	++++
4	1.62	1.12	1.65	1.42	3.85	0.95	1.28	2.56	1.90 ± 0.80	+
17	NT	100	100	100	100	100	100	100	100	++
18	53.7	>100	>100	>100	>100	>100	>100	>100	57.5	++
19	18.20	47.9	>100	25.1	>100	>100	>100	>100	64.6	0/+
20	>100	>100	>100	>100	>100	>100	>100	>100	>100	0
21	NT	2.45	6.17	6.61	5.89	11.0	4.47	7.08	6.17	++
22	<0.010	<0.010	2.69	0.30	2.63	0.023	2.04	3.02	0.525	0/+
23	5.62	6.46	NT	7.08	25.7	4.17	5.62	>100	9.77	+++
24	1.74	0.58	1.86	0.51	1.70	0.91	1.32	2.82	1.86	+++
25	89.1	60.3	>100	56.2	>100	>100	>100	>100	74.1	+++
26	52.50	>100	NT	83.2	>100	58.9	61.7	>100	74.1	++++
27	>100	36.3	85.1	29.5	81.3	93.3	>100	>100	67.6	++++
28	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	<0.010	0.014	0.033	+++
29	0.19	0.274	0.016	0.012	0.864	0.015	0.017	2.17	0.370 ± 0.28	++++
30	2.69	1.41	2.34	0.79	1.66	1.66	1.41	2.75	1.86	+++++

[a] The cytotoxicity GI_{50} 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. The activity of the compounds to produce Top1-mediated DNA cleavage was expressed semiquantitatively as follows: +: weak activity; ++ and +++: modest activity; ++++: similar activity as 1 μM camptothecin; +++++: greater activity than 1 μM camptothecin.

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.[23,24] The GI_{50} 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 GI_{50} for all of the cell lines tested (approximately 55) in which GI_{50} values below and above the test range (10^{-8}–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 activities of campothecin (**1**) and oracin (**4**) are 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 previously synthesized compounds are included in Table 1 for the sake of comparison with the newly reported Top1 inhibitors. These include the indenoisoquinolines **24**, **27**, and **30**, whose syntheses were also included in Scheme 3.[11]

In general, indenopyrans **17**, **18**, **19**, and **20** did not display potent cytotoxicity or Top1 inhibition in comparison to the corresponding indenoisoquinolines or camptothecin (**1**). However, this was to be expected since previous structure–activity relationships (SAR) determined have demonstrated the strong effect that prudent lactam substitution has on both cytotoxicity and Top1 inhibition. As supporting evidence, when the indenopyrans were converted to the corresponding indenoisoquinolines, Top1 inhibition improved for every compound and resulted in indenoisoquinolines **26**, **27**, **29**, and **30** demonstrating equal or superior Top1 inhibitory activity relative to camptothecin (**1**). An analogous result was also obtained regarding the cytotoxicities of the indenopyrans versus the indenoisoquinolines. Previous research indicated that an imidazole-substituted lactam provided potent cytotoxicity and Top1 inhibition (as realized from **30** and other undisclosed compounds).[11] Thus, it was not surprising that the two most cytotoxic new compounds synthesized (**28** and **29**) possessed an imidazole substituent.

When comparing compounds with identical lactam substitution, differences in the substitution of the isoquinoline 'A' ring typically exerted small fluctuations in cytotoxicity (within an order of magnitude) for all tested compounds and displayed little to no differences in Top1 inhibition. Compounds **22** and **28** are noteworthy exceptions that displayed potent cytotoxicity but slightly diminished Top1 inhibition within their respective series, suggesting that additional target(s) may be involved. Collectively, the results of this preliminary study were especially interesting since previous work had indicated substantial gains in biological activity for nitrated analogues[22] and a small, yet consistent, contribution to biological activity from di(methoxy)-substituents[11] when compared to analogues with unsubstituted 'A' rings. The increase was not seen in this series of analogues and allows one to speculate that the 'A' and 'D' rings may combine synergistically to account for the increased potency seen in previously synthesized analogues. From this limited series of compounds, it can also be inferred that lactam substitution has a profound impact on the biological activities of the indenoisoquinolines. This result highlights the strategy of synthesizing indenopyrans that can be diversely functionalized about the lactam position in a single step utilizing readily available primary amines in a convergent manner. Moreover, the indenopyrans provide obvious platforms for the parallel synthesis of indenoisoquinolines.

In conclusion, a one-pot synthesis of unsubstituted and substituted indenopyrans was developed and these compounds were utilized as intermediates for the synthesis of indenoisoquinolines displaying potent Top1 inhibition and in some instances increased cytotoxicity relative to previously reported compounds. The initial results of this study indicate that lactam substitution modulates the biological activity to a greater degree than modifications to the isoquinoline aromatic ring. However, the improved synthesis of indenopyrans represents a new synthetic strategy toward the development of Top1 anticancer agents.

Acknowledgments

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Supplementary data

Complete experimental procedures for the synthesis and characterization for all new indenoisoquinolines can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.008.

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